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TOXICITY OF MONOBASIC PHOSPHATES TOWARDS SOYBEANS GROWN IN SOIL- AND SOLUTION-CULTURES

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The mineral elements essential to the growth of plants in the soil or in culture media, may under certain conditions become extremely toxic to the plants. In a nutritive medium with a given total concentration of the necessary salt constituents for plants, marked changes in growth are sure to occur with any considerable alteration in the salt proportions. The salt proportions may be altered in such a manner as to produce considerable change in the growth rates without causing any specific injury to the plants. When, on the other hand, the physiological balance of the medium is too greatly disturbed by the alteration of the salt proportions, toxic effects in some specific form are quite sure to appear, and these can usually be related to one or more of the constituents of the medium. Thus, in a series of 84 four-salt nutritive solutions, all having the same total concentration but with varying proportions of the same four salts, Tottingham (14) found that a number of his solutions caused specific injury to wheat seedlings, while other solutions of the same series produced excellent growth without toxic effects. Tottingham was able to relate the injury to the magnesium content of the solutions. He pointed out, however, that the conditions determining the degree of the injury were related to the relative proportions of all the constituents rather than to the partial concentration of magnesium alone. In a more recent investigation (12) of the same character, with solutions comprising the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulfate, in 36 different proportions but with constant total concentrations, it was found that out of the total of 36 solutions, 7 caused very severe injury to wheat seedlings and 14 produced slight injury. The remaining 15 solutions showed various degrees of productiveness without toxic effects. The disturbance here observed was practically identical with that described by Tottingham (14) and called by him magnesium injury.

In a nutritive medium with constant relative proportions of the necessary salt constituents for plants, variation in the growth rates without toxic effects may be brought about also by alterations in the total concentration of the medium. This is primarily an osmotic relation and appears to be dependent upon the ease with which water may be taken into the cells. Trelease (15) has shown that the growth rate (as indicated by dry-weight yields) is an ap-

proximate linear relation to the concentration of the medium above the optimum, the growth rate decreasing with an increase in concentration. If, however, the total concentration of the medium is gradually increased without alteration of the salt proportions, a point is finally reached where the plants may show symptoms of specific poisoning due to the absorption of excessive amounts of one or more of the essential constituents. This has been shown to occur with total concentrations not above the optimum for plant growth. Tottingham's series of 4-salt solutions with a total osmotic concentration value of 0.5 atmospheres produced no magnesium injury to wheat seedlings. When the concentration of the solutions was increased to an osmotic value of 2.5 atmospheres and 8.15 atmospheres, many of the solutions produced severe injury. The solutions of the 3-salt series employed by the present writer (12) caused no toxic symptoms in wheat seedlings when the total osmotic concentration value was 0.10 atmospheres, but with the same relative salt proportions at total concentrations having osmotic values of 1.75 atmospheres and 4 atmospheres, many of the solutions produced severe injury.

In the course of some experimental work (13) concerning the influence of various salts on the growth of soybeans in soil, it was observed that several phosphate salts, when added singly to the soil, even in comparatively small applications, proved to be toxic to the plants, which not only were retarded in their development, but also suffered specific injury. It was the purpose of the present investigation to study somewhat in detail the nature of this injury and to determine the general effects of several monobasic phosphate salts on the growth of soybeans under various experimental conditions. These tests involved three distinct sets of experiments employing five different monobasic phosphate salts: (a) in soil to which the salts were added singly in the form of solutions with varying concentrations; (b) in connection with a complete fertilizer ration added to the soil in mixed solutions with constant total concentrations; and (c) in culture solutions with complete nutrient mixtures.

EXPERIMENTAL METHODS

1. Preparation of stock solutions

Throughout these tests Baker's analyzed chemicals were used, including mono-sodium phosphate, mono-ammonium phosphate, mono-potassium phosphate, mono-calcium phosphate, mono-magnesium phosphate, and magnesium sulfate in the crystalline form, and calcium nitrate in the form of fused lumps (labeled as containing $4H_2O$). In all the work with soil cultures, the salts were introduced into the soil in the form of solutions. Stock solutions of the salts, except mono-calcium phosphate, were prepared by dissolving separately in volumetric flasks a gram molecular weight of each salt, after which the solutions were made up to liter volume at 15°C. The distilled water used throughout was obtained from a "Barnstead" still and was stored in large glass bottles, from which it was drawn for immediate use. The stock solution of mono-cal-

cium phosphate was prepared by dissolving one-eighth (0.125m) gram molecular weight of the salt in a volumetric flask, keeping the mixture at a temperature below 15°C. until the process of solution was completed, in order to avoid decomposition and precipitation, which sometimes occurred when the solutions were prepared at room temperature. The flask was then filled to liter volume at 15°C.

For immediate use, the volume-molecular stock solutions were diluted to concentrations better suited to the preparation of the culture solutions. The concentration of the diluted stock solutions was one-fourth (0.25m) molecular, excepting the mono-calcium phosphate, which had a concentration of one-eighth (0.125m) molecular.

Throughout this work all glass vessels employed as containers of solutions or distilled water were thoroughly washed with bichromate-sulfuric acid cleansing mixture, and were then rinsed with tap water, followed by distilled water.

2. *Preparation of culture solutions*

a. Pure solutions. Throughout this study concentrations will be stated in terms of gram molecules per liter of solution (volume-molecular concentration) or in terms of possible osmotic pressure in atmospheres (osmotic concentration).

Five series of pure solutions corresponding to the five phosphate salts, were employed in soil cultures. Each series comprised 10 solutions. Eight solutions of each series, beginning with the first, varied in osmotic concentration from 0.5 atmospheres to 4 atmospheres, by increments of 0.5 atmospheres. The remaining two solutions of each series had concentrations of 5 and 7 atmospheres, respectively. Each series possessed, therefore, a range in concentration from an osmotic value of 0.5 atmospheres to an osmotic value of 7 atmospheres.

In table 1 are given the approximate osmotic concentration values in atmospheres, and the volume-molecular concentrations of the ten solutions of each series, as well as the per cent of total salt added to each soil culture, calculated on the basis of the weight of air-dry soil, which in all cases was 4 kgm. to each culture.

The osmotic concentration values of these solutions were calculated approximately according to the method employed by Tottingham (14) who based his calculations on the data of electrolytic dissociation as given by Jones (7). Electrolytic dissociation data for the phosphates here employed are not found in Jones' tables. The data required for the calculation of the osmotic concentration values of mono-potassium phosphate are given by Tottingham (14, p. 177), and similar data for mono-sodium and mono-ammonium phosphates are given by Watkins and Jones (16). Data for mono-calcium and mono-magnesium phosphates were not available. The osmotic concentrations of these salts in solutions were determined approximately by the method of cryoscopy (11).

b. Mixed solutions. Five series of mixed solutions were employed. Each of these consisted of the Tottingham (14) series of nutrient solutions in which a monobasic phosphate was substituted for the potassium nitrate. The series will be designated series I, II, III, IV, and V, and will correspond to the salts mono-sodium phosphate, mono-ammonium phosphate, mono-potassium phosphate, mono-calcium phosphate, and mono-magnesium phosphate, respectively. Thus each solution of a series contained, in addition to the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulfate, one of the five monobasic salts. The three salts, mono-potassium phosphate, calcium nitrate and magnesium sulfate, containing all the so-called essential constituents (excepting iron) of a complete nutrient mixture, were present in

TABLE 1

Approximate osmotic and volume-molecular concentrations of solutions of monobasic phosphates employed singly in soil cultures; also amount of salts in each culture expressed as per cent of the weight of air-dry soil

SOLUTION NUMBER	OSMOTIC CONCENTRATION	VOLUME-MOLECULAR CONCENTRATIONS					WEIGHT OF SALT AS PER CENT OF AIR-DRY SOIL				
		Series I NaH_2PO_4	Series II $(NH_4)H_2PO_4$	Series III KH_2PO_4	Series IV $CaH_4(PO_4)_2$	Series V $MgH_4(PO_4)_2$	Series I NaH_2PO_4	Series II $(NH_4)H_2PO_4$	Series III KH_2PO_4	Series IV $CaH_4(PO_4)_2$	Series V $MgH_4(PO_4)_2$
	atm.										
1	0.5	0.0100	0.0101	0.0104	0.0071	0.0076	0.015	0.015	0.018	0.021	0.021
2	1.0	0.0201	0.0204	0.0210	0.0143	0.0153	0.030	0.030	0.036	0.042	0.042
3	1.5	0.0305	0.0308	0.0321	0.0216	0.0231	0.046	0.044	0.055	0.063	0.063
4	2.0	0.0425	0.0426	0.0442	0.0286	0.0306	0.064	0.063	0.075	0.084	0.084
5	2.5	0.0539	0.0539	0.0560	0.0359	0.0384	0.081	0.077	0.095	0.105	0.105
6	3.0	0.0658	0.0658	0.0685	0.0431	0.0461	0.099	0.095	0.116	0.126	0.125
7	3.5	0.0769	0.0769	0.0800	0.0504	0.0540	0.115	0.111	0.136	0.148	0.147
8	4.0	0.0901	0.0900	0.0936	0.0578	0.0618	0.135	0.129	0.159	0.169	0.168
9	5.0	0.1132	0.1130	0.1176	0.0805	0.0860	0.170	0.162	0.199	0.236	0.234
10	7.0	0.1622	0.1615	0.1685	0.1051	0.1120	0.243	0.232	0.281	0.307	0.306

corresponding solutions of the five series in the same osmotic and volume-molecular proportions. The different solutions all had approximately the same total osmotic concentration (2.5 atmospheres), but each solution of the same series differed from all the others in the relative proportions of the four component salts.

In addition to these five series, a sixth series was employed as a control series; this, for the sake of convenience, may be called the 3-salt series. In the preparation of the 3-salt series, mono-potassium phosphate, calcium nitrate, and magnesium sulfate only were employed. These three salts were present in each solution of this series in the same relative proportions as were the same three salts in corresponding solutions of the other five series. However, the total osmotic concentrations of the solutions of the 3-salt series were

made equal to those of the solutions of the other five series (2.5 atmospheres), each containing four constituent salts. Thus the partial volume-molecular concentrations of the three salts in each solution of the 3-salt series differed from the partial volume-molecular concentrations of the same salts in corresponding solutions of the other series, but the relative proportions of these three salts, in corresponding solutions of the different series, remained the same.

The method here adopted to control the osmotic concentrations was the same as that employed by Tottingham (14, p. 192). The relative proportions of the salts were varied in such a way that a decrease in the partial concentration of one salt was balanced by a sufficient increase in the partial concentrations of the remaining salts to keep the total osmotic concentration of the solution constant. The total osmotic concentration (2.5 atmospheres) for each 4-salt series was considered to be divided into ten equal parts and these parts were distributed among the four salts in all possible proportions. Thus each salt produced in the different solutions of a series, from one-tenth to seven-tenths of the total osmotic concentration.

A detailed discussion of the methods by which the osmotic concentration of a series of solutions, differing in the proportions of the constituent salts, may be controlled, as well as a discussion of the methods of calculation by which the partial osmotic concentrations may be approximately determined for complex mixtures with constant total osmotic concentrations, has been presented by Tottingham (14, p. 177–182, 192).

Variations in the proportions of the four salts, as here employed, by increments of one-tenth of the total osmotic concentration yield a series of 84 solutions. Six such series, therefore, comprise 504 solutions. It was found impracticable to conduct so large a number of cultures during the same time period. In the present work, therefore, the number of solutions employed was reduced to 20 in each series. These were selected to correspond to 20 representative solutions from the Tottingham (14) series of 84 solutions As is well known, the scheme adopted by Tottingham to represent diagrammatically the relative composition of the solutions of his 4-salt series, makes use of six triangles (T_1, T_2, T_3, etc.) and a point (T_7). The rows of each triangle are numbered from the base to the apex (R_1, R_2, R_3 etc.) and the solutions or cultures in each row are numbered from left to right (C_1, C_2, C_3, etc.), A detailed description of this arrangement is presented by Tottingham (14, p. 192–195) and need not be repeated here. The twenty cultures of the Tottingham series corresponding to those of each series here employed, were selected in the following manner. From the Tottingham diagrammatic scheme the three triangles T_2, T_4, and T_6 were omitted, leaving the three triangles T_1, T_3, and T_5 and the point T_7. By omitting from these remaining three triangles every other row, starting with the second row from the base of each triangle, and by further omitting every other solution from the remaining rows, starting with the second solution in each row, a total of twenty, out of

the entire series of 84 solutions, remain. These twenty solutions are uniformly distributed throughout the series.

The first column of table 2 presents the solution numbers as they occur in the Tottingham series. It will be observed that the triangles, rows, and cultures represented by even numbers are omitted while all those represented by odd numbers are retained and appear in the table. The table gives the partial volume-molecular concentration of each solution of the five series. In the last three columns are given the partial volume-molecular concentrations of the three salts KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, as they were employed with each of the five phosphate salts to form the five different series. Thus, the four salts employed in series I were present in the various solutions in the partial volume-molecular concentrations as given in the second column for NaH_2PO_4, and in the last three columns for KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, respectively. In the same manner are given successively the partial volume-molecular concentrations of the solutions of series II, III, IV and V.

TABLE 2

Partial volume-molecular concentrations of five monobasic phosphates each employed with KH_2PO_4 $Ca(NO_3)_2$ *and* $MgSO_4$ *to form five series of culture solutions with total osmotic concentrations of 2.5 atmospheres*

SOLUTION NUMBER	PARTIAL CONCENTRATIONS, VOLUME-MOLECULAR							
	Series I NaH_2PO_4	Series II $(NH_4)H_2PO$	Series III KH_2PO_4	Series IV $CaH_4(PO_4)_2$	Series V $MgH_4(PO_4)_2$	Component salts of each solution, series I to V		
						KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
$T_1R_1C_1$	0.0050	0.0051	0.0052	0.0036	0.0038	0.0052	0.0036	0.0406
C_3	0.0050	0.0051	0.0052	0.0036	0.0038	0.0052	0.0108	0.0290
C_5	0.0050	0.0051	0.0052	0.0036	0.0038	0.0052	0.0180	0.0174
C_7	0.0050	0.0051	0.0052	0.0036	0.0038	0.0052	0.0252	0.0058
R_3C_1	0.0050	0.0051	0.0052	0.0036	0.0038	0.0156	0.0036	0.0290
C_3	0.0050	0.0051	0.0052	0.0036	0.0038	0.0156	0.0108	0.0174
C_5	0.0050	0.0051	0.0052	0.0036	0.0038	0.0156	0.0180	0.0058
R_5C_1	0.0050	0.0051	0.0052	0.0036	0.0038	0.0260	0.0036	0.0174
C_3	0.0050	0.0051	0.0052	0.0036	0.0038	0.0260	0.0108	0.0058
R_7C_1	0.0050	0.0051	0.0052	0.0036	0.0038	0.0364	0.0036	0.0058
$T_3R_1C_2$	0.0150	0.0153	0.0156	0.0108	0.0114	0.0052	0.0036	0.0290
C_3	0.0150	0.0153	0.0156	0.0108	0.0114	0.0052	0.0108	0.0174
C_5	0.0150	0.0153	0.0156	0.0108	0.0114	0.0052	0.0180	0.0058
R_3C_1	0.0150	0.0153	0.0156	0.0108	0.0114	0.0156	0.0036	0.0174
C_3	0.0150	0.0153	0.0156	0.0108	0.0114	0.0156	0.0108	0.0058
R_5C_1	0.0150	0.0153	0.0156	0.0108	0.0114	0.0260	0.0036	0.0058
$T_5R_1C_1$	0.0250	0.0255	0.0260	0.0180	0.0190	0.0052	0.0036	0.0174
C_3	0.0250	0.0255	0.0260	0.0180	0.0190	0.0052	0.0108	0.0058
R_3C_1	0.0250	0.0255	0.0260	0.0180	0.0190	0.0156	0.0036	0.0058
$T_7R_1C_1$	0.0350	0.0357	0.0364	0.0252	0.0266	0.0052	0.0036	0.0058

It will be observed that the solutions of series III contain only the three salts, KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, but the partial osmotic concentration due to the KH_2PO_4 in each solution of this series is the same as that supplied by the total phosphates in the corresponding solutions of the other four series. The solutions of series III, therefore, will be treated throughout the study as though they were 4-salt solutions.

Table 3 gives the partial volume-molecular concentrations of the solutions of the 3-salt series. It is to be noted that while the relative proportions of the salts in each solution represented in this table are the same as are those of

TABLE 3

Partial volume-molecular concentrations of KH_2PO_4, $Ca(NO_3)_2$, *and* $MgSO_4$ *in culture solutions having total osmotic concentration of 2.5 atmospheres (3-salt series)*

SOLUTION NUMBER	PARTIAL CONCENTRATIONS, VOLUME-MOLECULAR		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
$T_1R_1C_1$	0.0058	0.0040	0.0451
C_3	0.0058	0.0120	0.0322
C_5	0.0058	0.0200	0.0193
C_7	0.0058	0.0280	0.0064
R_3C_1	0.0173	0.0040	0.0322
C_3	0.0173	0.0120	0.0193
C_5	0.0173	0.0200	0.0064
R_5C_1	0.0289	0.0400	0.0193
C_3	0.0289	0.0120	0.0064
R_7C_1	0.0404	0.0040	0.0064
$T_3R_1C_1$	0.0074	0.0051	0.0414
C_3	0.0074	0.0154	0.0248
C_5	0.0074	0.0257	0.0083
R_3C_1	0.0223	0.0051	0.0248
C_3	0.0223	0.0154	0.0083
R_5C_1	0.0371	0.0051	0.0083
$T_5R_1C_1$	0.0104	0.0072	0.0348
C_3	0.0104	0.0216	0.0116
R_3C_1	0.0312	0.0072	0.0116
$T_7R_1C_1$	0.0173	0.0120	0.0193

the same three salts [KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$] in the corresponding solutions of table 2, the partial volume-molecular and partial osmotic concentrations are not the same, since the total osmotic concentration (2.5 atm.), which is the same for the solution of all the series, is made up of three salts in the solutions of this one series, and of four salts in the solutions of each of the other five series, except series III.

In preparing the individual culture solutions, the required amount of stock solution of the phosphate salt to be employed was drawn from a burette into a 500-cc. volumetric flask partially filled with water. To this was added in a

similar manner the required amounts of the stock solution of mono-potassium phosphate, calcium nitrate, and magnesium sulfate, in the order given. The flask was then filled to volume with distilled water.

3. The soil cultures

The soil employed throughout this work consisted of a mixture of white quartz sand and a rich sandy loam. The latter was obtained from the experiment grounds of the botanical department of the New Jersey Agricultural Experiment Station. The soil and sand were air-dried separately, after which they were sifted through a wire screen of 2-mm. mesh, and a sufficient quantity of each was stored for use during the work. Equal parts by volume of the air-dry soil and sand, measured without any tapping or packing, were placed in a rectangular galvanized sheet-iron pan, 110 x 65 x 20 cm., and were thoroughly mixed. A sufficient quantity of this mixture was prepared at one time to provide for all the cultures to be conducted during the same time period. This procedure secured an approximately uniform soil medium for all the cultures and yielded a soil possessing a water-holding capacity of 28.2 per cent (average of several determinations) of its dry weight, determined according to the method of Hilgard (5).

The containers employed for the soil cultures consisted of stone-ware jars, glazed inside and outside, each with a capacity of 4 liters, and with an inside diameter of approximately 16 cm.

Each soil culture was prepared separately. Four kilograms of the air-dry soil-sand mixture was placed in a mixing pan of galvanized sheet iron. To the dry soil was now added 500 cc. of solution which was then thoroughly mixed with the soil, after which the preparation was placed in a container and the whole weighed to the nearest gram.

The addition of 500 cc. of solution to each 4 kgm. of air-dry soil produced a soil moisture content approximately 12.5 per cent of the weight of air-dry soil, or 44.3 per cent of the water-holding capacity of the soil.

The soybeans used were of the "Wilson" variety. The seed was obtained from the New Jersey Agricultural Experiment Station, having been raised on the experimental grounds of the botanical department of the station. Seeds selected for uniformity of size were germinated in moist sand contained in galvanized sheet-iron pans. The pans stood in the experiment greenhouse until the seedlings were about 4 cm. tall. Vigorous seedlings, nearly equal in size, were selected, carefully removed from the moist sand, and transplanted to the pots containing the soil cultures previously prepared. Five seedlings were thus transplanted to each pot.

To prevent loss of water by evaporation during the growth period, each pot was sealed by pouring over the surface of the soil around the seedlings, melted paraffine wax prepared according to the formula of Briggs (2) and Shantz. The melting point of the wax used was about 40°C. For the purpose of watering

the plants, a paper funnel, partially buried in the soil in the inverted position, was placed in each pot. The funnel extended about one-fourth the distance to the bottom of the pot, its upper end projecting through the wax seal at the soil surface. These funnels, approximately 8 cm. long, with openings of 5 cm. and 1.5 cm. in diameter, were made of heavy wrapping paper and were then thoroughly saturated with melted paraffine to render them impervious to moisture. The upper opening of each funnel was kept tightly closed with a paraffined cork stopper, except for a short period at the time of each watering. Each culture was weighed immediately preceding and directly following the placing of the funnel and the application of the wax seal. The difference in weight was added to the original weight of the container.

The plants were watered every second day during the early stages of growth, and every day during the later stages. At each watering the cultures were weighed and a sufficient amount of distilled water was poured through the funnel of each culture to restore it to its original weight.

At the end of the growing period the plants were cut just above the wax seal. The tops from each culture were placed in a weighing bottle and dried to constant weight at a temperature of about 103°C., and the dry weight obtained.

4. The water cultures

The solutions which were employed in the preparation of the soil cultures above described, were used also in experiments with water cultures. The solutions were prepared according to the formulae given in tables 2 and 3, and were used without alteration, except that to each solution was added a trace of iron in the form of ferric phosphate.

The containers employed consisted of pint "Mason" jars which had previously been thoroughly cleaned with bichromate-sulfuric acid cleaning solution and rinsed with distilled water. With each culture 500 cc. of solution were employed. The soybeans used were of the same variety and were obtained from the same lot as were those used in connection with the soil cultures. The seeds were germinated in moist sphagnum. Selected seedlings about 5 cm. tall were mounted in thoroughly paraffined flat cork stoppers in a manner similar to that described by Tottingham (14, p. 173–175). The stoppers were then placed in the culture jars. Each culture comprised three plants. To exclude the light from the roots of the plants, the culture jars were covered with cylindrical paper shells which were black on the inside and nearly white on the outside. These shells were prepared in a manner similar to that described by the writer (12, p. 344). The solutions were renewed every four or five days.

At the end of the growth period the tops were severed from the roots just at the lower surface of the cork stoppers. The tops were then dried and the dry weights obtained in the same way as were the yields from the soil cultures.

During the periods of growth the general character of the development of the tops in the soil cultures, as well as of the tops and roots in the water cul-

tures, was noted. The characteristics of the injury sustained by the plants of the various culture groups were studied, and the injured plants were compared from time to time with others of the same group and also with the injured plants of the other groups.

EXPERIMENTAL RESULTS

I. Introductory

In the following sections are presented the results of three distinct experiments, each experiment consisting of two series of cultures, of which the second series is a repetition of the first. For the sake of convenience, the two series of each experiment will be designated series A and series B throughout. A comparison will be given of the physiological effects upon the growth of soybeans, produced by the various cultures (of each series) containing the soluble phosphate salts: (a) when these salts were added singly to soil cultures; (b) when added to soil cultures in nutrient mixtures, and (c) when employed in nutrient solutions as water cultures. The responses of the plants to the media in which they grew will be studied with reference to the total yields of dry tops, and especially with respect to the toxic influences of the various salts resulting in any unusual pathological conditions, as these might be detected by general observation.

II. Experiment I. Monobasic phosphate salts singly in soil cultures

In this experiment five groups of cultures were employed, the five groups corresponding to the five different salts, NaH_2PO_4, $(NH_4)H_2PO_4$, KH_2PO_4, $CaH_4(PO_4)_2$, and $MgH_4(PO_4)_2$. Each group comprised 11 cultures, as previously described, including one check culture which received no salt. The substratum in which the plants grew was prepared by thoroughly mixing 500 cc. of pure solution of the salt in question with 4 kg. of air-dry soil-sand mixture. The total amount of salt received by each of these cultures was included in this initial application of 500 cc. of solution. The approximate osmotic values (atm.) of the ten solutions of each series added to the soil cultures, their volume-molecular concentrations, and the weight of the salt in each culture expressed in per cent of the weight of the air-dry soil are given in table 1. Series A of this experiment was conducted from August 25 to October 4, 1916; series B was carried out between February 24 and April 5, 1917.

1. Dry weights. The numerical data of the yields of soybean tops from the cultures of these two series are presented in table 4. The table gives the dry weights of tops for each of the five groups of cultures of series A and of series B. The dry-weight yield for each culture represents the average for six plants. The first column of the table gives the approximate osmotic concentration values of the solutions applied to the cultures. Then follow five sec-

tions with three columns in each section, referring to the dry weights of tops, each section giving the data for a single group. The first two columns in each section present the data for series A and B, respectively, and the third column in each section gives the value obtained by averaging the corresponding data from series A and B. Each of these averages thus represents the data of the two series combined. Each of the dry-weight values is expressed in terms of the corresponding value of the check culture considered as unity, but the actual dry weight of this culture is given in parenthesis in grams, so that the actual weight for any culture may be obtained by multiplying its relative weight by the actual weight of the check culture. The actual average values of the check cultures for series A and B, as given in the first two columns of

TABLE 4

Relative dry weights of soybean tops grown 40 days in soil cultures prepared with pure solutions of monobasic phosphate salts of various concentrations: Series A, conducted from August 25 to October 4, 1916; Series B, from February 24 to April 5, 1917

OSMOTIC CONCENTRATION	GROUP I (NaH_2PO_4)			GROUP II ($NH_4H_2PO_4$)			GROUP III (KH_2PO_4)			GROUP IV [$CaH_4(PO_4)_2$]			GROUP V [$MgH_4(PO_4)_2$]		
	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average
atm.															
0.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	5.91	7.57	6.74	5.91	7.57	6.74	5.91	7.57	6.74	5.91	7.57	6.74	5.91	7.57	6.74
0.5	0.71	1.01	0.86	0.87	0.73	0.80	0.95	0.93	0.94	0.82	0.80	0.81	0.73	0.96	0.85
1.0	0.59	0.95	0.77	0.85	0.68	0.76	0.85	0.87	0.86	0.81	0.78	0.80	0.70	0.95	0.83
1.5	0.46	0.91	0.69	0.73	0.56	0.66	0.78	0.80	0.79	0.35	0.75	0.55	0.65	0.73	0.69
2.0	0.31	0.79	0.55	0.50	0.41	0.46	0.76	0.77	0.77	0.31	0.58	0.45	0.64	0.53	0.59
2.5	0.26	0.62	0.44	0.32	0.41	0.37	0.75	0.58	0.67	0.30	0.54	0.42	0.48	0.45	0.47
3.0	0.25	0.48	0.37	0.27	0.37	0.32	0.57	0.49	0.53	0.27	0.53	0.40	0.48	0.37	0.43
3.5	0.25	0.42	0.34	0.22	0.28	0.25	0.57	0.43	0.50	0.25	0.41	0.33	0.37	0.35	0.36
4.0	0.23	0.33	0.28	0.18	0.28	0.23	0.54	0.36	0.45	0.22	0.32	0.27	0.32	0.34	0.33
5.0	0.19	0.26	0.23	0.16	0.18	0.17	0.43	0.36	0.40	0.11	0.28	0.20	0.28	0.34	0.31
7.0	0.13	0.24	0.19	0.15	0.15	0.15	0.36	0.35	0.36	0.05	0.26	0.16	0.14	0.23	0.19

each section in the table, were obtained by averaging the yield values of the check cultures from the five groups.

The average relative dry-weight values of each group form a somewhat uniformly decreasing series of numbers. These were plotted to form the graphs of figure 1. Here the ordinates represent the relative dry-weight values, while the abscissas represent the approximate osmotic concentration values (atmospheres) of the single-salt solutions added to the cultures of each group. Since the osmotic concentrations of the solutions added to corresponding cultures of the five groups were approximately the same, the average dry weight values for the five groups were plotted with the same abscissas. The average relative yield from the check cultures is here represented graph-

ically by a horizontal broken line and the actual value in grams is given at the right.

The relative degrees of injury sustained by the plants of the various cultures of each group are graphically represented in figure 1 by the broad vertical lines just below the graph representing the yields of the group in question. The shortest vertical line represents slight injury, vertical lines of medium length indicate pronounced injury, while the longest lines denote severe injury. The relative terms, *slight*, *pronounced*, and *severe*, will be defined later in connection with the description of the injury produced by the salts.

From the graphs of figure 1 and from the data of table 4, it is at once clear that the growth of soybean tops was very unfavorably influenced by each of the monobasic phosphate salts applied singly to the soil in which the plants were rooted. Each of the graphs slopes uniformly downward to the right, denoting a decrease in the yield of dry tops with each increase in the application of the salt.

As indicated by these graphs, the average yield from the check cultures was considerably higher than that of the highest average yield from any culture of the five groups here employed. Relative to the average yield from the check cultures considered as 1.00, the highest average yield from each of the five series is as follows: series I [NaH_2PO_4], 0.86; series II [$(NH_4)H_2PO_4$], 0.80; series III [KH_2PO_4], 0.94; series IV [$CaH_4(PO_4)_2$], 0.81; series V [$MgH_4(PO_4)_2$], 0.85. The lowest average yields from the five series, taken in the same order as given above are: 0.19, 0.15, 0.36, 0.16, and 0.19. Group III here shows the highest average relative dry-weight values throughout the entire series. The average yields from corresponding cultures of group I and group V are nearly equal and are considerably lower in value than are the average dry weights from corresponding cultures of group III, showing the highest average yields, while group II shows the lowest average yields. It is thus clear that mono-potassium phosphate, aside from any specific injury to the plants, has the least unfavorable influence upon the growth of soybean tops, while mono-ammonium phosphate is the most unfavorable of the five salts, as these were here employed.

2. Injury. As was to be expected, the growth of the plants in those cultures of each group to which were applied solutions having concentrations with osmotic values between 2.5 and 7.0 atmospheres, was soon visibly retarded. This retardation in growth was followed by specific injury to the plants, appearing first in the cotyledons in the form of dark brown discolorations around the margins. In severe injury the discoloration spread rapidly over the whole surface of the cotyledons, and this was followed soon after by death and falling of the organs. The injury next appeared on the foliage leaves. Usually an interval of several days elapsed between the time when the injury first became apparent on the cotyledons and the time of its appearance on the foliage leaves. Injury to the leaves appeared first on or near the margins, in the form of small, yellowish, round spots, which rapidly assumed the

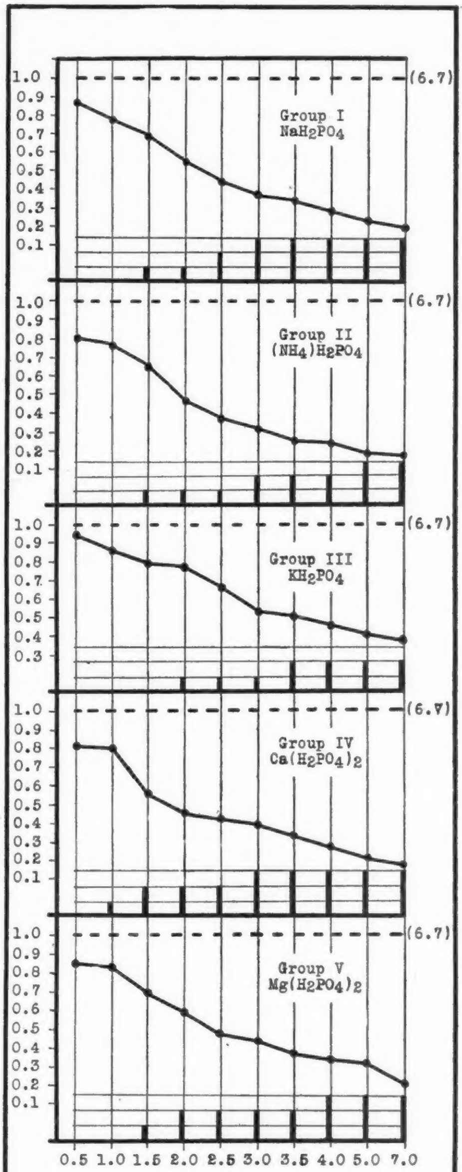

FIG. 1. RELATIVE DRY WEIGHTS AND INJURY OF SOYBEAN TOPS GROWN IN SOIL CULTURES CONTAINING PHOSPHATES SINGLY

characteristic dark brown coloration. In cases of severe injury the affected portions of the leaves gradually increased in size until the injury had spread to the entire leaf surface. This kind of injury was invariably followed by death and falling of the leaves affected. The injury spread, in severe cases, to include all the leaves of the plant. In the later stages of growth, the stems also were affected. This occurred frequently when the leaves of the affected plant were only slightly injured. Injury to the stem appeared in the form of oblong brown spots which gradually increased in size, with shrinking of the tissues.

The cultures of each group may be divided according to the degrees of injury sustained, into four classes: (a) those without specific injury; (b) those which exhibited *slight* injury, not sufficiently severe to cause falling of the leaves affected; (c) those which sustained *pronounced* injury, severe enough to cause the leaves to fall but not sufficiently severe to cause the death of the plants before the end of the growth period; (d) those which sustained *severe* injury resulting in the death of the plants before the end of the growth period. As previously stated, the relative degrees of injury are graphically represented in figure 1 by broad vertical lines just below the graph representing the dry-weight yields of the series in question.

A comparison of the diagrams representing the relative degrees of injury sustained by the cultures of the various groups, brings out the fact that the total injury exhibited by group III [KH_2PO_4] was less than that sustained by any of the other groups. This group showed four cultures with pronounced injury and three with slight, leaving three uninjured cultures. Group IV [$CaH_4(PO_4)_2$], on the other hand, exhibited the greatest total injury of any group. As indicated by the diagram, five cultures in this group were killed before the end of the growth period, three sustained pronounced injury, and one exhibited slight injury, leaving only one uninjured culture in the entire group. Each of the remaining three groups (I, II and V) possessed two cultures which remained uninjured throughout the entire period. Groups I and IV each possessed five cultures which sustained severe injury resulting in the death of the cultures, and these were corresponding cultures of the two groups.

From these considerations it is clear that the salt KH_2PO_4, (group III), as here used, is less pronounced in its capacity to produce specific injury to soybean tops than are any of the other salts here employed. Mono-calcium phosphate (group IV), on the other hand, shows a more pronounced harmful influence with respect to the injury observed, than do any of the other salts.

A careful comparison of the injured plants showed that the nature of the injury sustained by the cultures of the various groups was identical. The only apparent differences, apart from size, between the plants from the cultures of the different groups were the degrees of the injury sustained. It is to be concluded, therefore, that the injury to the plants resulting from the presence of the phosphate salts, as these were here employed singly, was due to

some property common to all the solutions used. The injury cannot be the result of mere physical concentration, since it occurred in plants of those cultures to which were applied solutions having concentrations with no greater osmotic values than from 1 to 2.5 atmospheres: concentrations which have repeatedly been shown to be not above the optimum for good plant growth. Moreover, the concentrations of these solutions cannot be supposed to remain unchanged upon being introduced into the soil, since the phosphates when applied to the soil in soluble forms are undoubtedly, to a large extent at least, eventually precipitated in combination with various bases existing in the soil. That the injury is accentuated by excessive concentration, is clearly evident from the graphs of figure 1.

It thus appears that the specific injury here observed can not be attributed to the influence of the cations, for it is scarcely to be supposed that each of the five ions, Na, NH_4, K, Ca and Mg, could affect the plants alike in this respect. The injury, therefore, must be related to the common atomic group H_2PO_4, or to the ions resulting from the partial dissociation of this group in the soil solution. This conclusion is in harmony with that of Harris (4) who found that to the anion more than to the cation, is to be attributed the toxic effects of the alkali salts in the soil. That the H-ion concentration of these monobasic phosphates singly in solutions is comparatively high, is of course, well understood, and the injury here observed is undoubtedly related to the H-ion concentration in the soil solution.

III. Experiment II. Monobasic phosphate salts in soil cultures with complete nutrient mixtures

In this experiment six groups of cultures were employed, corresponding to the six series of mixed solutions whose formulas are presented in tables 2 and 3. The tables give the approximate partial volume-molecular concentrations of the solutions as these were here used. Each solution of the six series had a total osmotic concentration value of approximately 2.5 atmospheres. As described in a former section, the soil cultures of each group were prepared by mixing separately 500 cc. of each solution of the series in question with 4 kgm. of air-dry soil. The total amount of salts added to each culture was included in this initial application of 500 cc. of the mixed solution. Each group comprised 21 cultures, including one check culture, which was prepared in the same way as were the other cultures, except that the dry soil received an initial application of 500 cc. of distilled water instead of solution.

During the growth period the cultures stood in parallel rows (20 cultures in a row) on central tables in the experimental greenhouse, and were frequently shifted in their positions according to a definite plan. Series A of this experiment was conducted from November 5 to December 5, 1916. Series B was exactly like series A, but was carried out between January 10 and February 9, 1917.

1. Dry weights. The numerical data of the yields of tops are presented in table 5. The table comprises six sections, each of which refers to the data of a single group. The first two columns in each of the six sections give the dry weights of tops relative to the average dry weight of the six check cultures for series A and B, respectively. The third column of each section gives the average dry-weight yield for series A and series B together. The average absolute dry weights of the check cultures for series A and B are given in parenthesis. The culture numbers given in the first column of the table refer to the positions occupied in the Tottingham series by the solutions employed in the preparation of the soil cultures.

To bring out the relation between the salt proportions and the relative dry yields of tops of the various groups, the data of table 5 are graphically represented in figure 2. To prepare these graphs, the average relative dry weights of tops of the 3-salt group were first arranged in the order of their magnitudes, beginning with the highest, to form a somewhat uniformly decreasing series of numbers. The relative yields of each of the other five groups were then arranged in the same order, after which they were plotted to form the five graphs of figure 2, shown as the irregular dotted lines. The abscissas were here arbitrarily chosen to represent the cultures, the numbers of which, being the same for corresponding cultures of each of the five groups, are placed below. The ordinates represent the average relative dry-weight values. The average relative yield from the check culture is represented by a horizontal broken line, and the actual average value in grams is given at the right. As in figure 1, the broad vertical lines just below each set of graphs indicate the relative degrees of injury sustained by the plants of the cultures indicated. The numbers at the top indicate the number of tenths of the total osmotic concentration due to the total phosphates in the solutions whose numbers are given below.

In order to bring out more clearly the effect upon the growth of soybean tops of an additional application of the monobasic phosphate salts to soil cultures containing a complete fertilizer, the graph representing the average relative dry weights of tops of the 3-salt group, arranged in the descending order of their values, was superimposed upon each of the other graphs, using the same scale of ordinates. This graph exhibits a uniformly downward slope to the right, as was previously arranged.

Each graph of figure 2 has a decided tendency to slope downward to the right, but the graph of each group except group V crosses the graph of the 3-salt group at various points, but chiefly in its lower portions, thus indicating that many of the poorer 3-salt proportions were improved for the growth of soybean tops by the presence of the additional monobasic phosphate salt. The best set of salt proportions of the 3-salt group, however, was decreased in productiveness by the presence of each one of the phosphate salts except monocalcium phosphate (group IV). The maximum average yield of group IV slightly exceeds that of the 3-salt group, the former having a value of 1.63 as compared with the average yield of the check cultures, while the latter has a

TABLE 5

Relative dry weights of soybean tops grown thirty days in soil cultures prepared with mixed solutions having total osmotic concentration values of approximately 2.5 atmospheres. Series A, conducted from November 5 to December 5, 1916; Series B, from January 10 to February 9, 1917

CULTURE NUMBER	GROUP I			GROUP II			GROUP III			GROUP IV			GROUP V			3-SALT GROUP		
	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average
Check	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2.12	2.76	2.442	2.12	2.76	2.442	2.12	2.76	2.442	2.12	2.76	2.442	2.12	2.76	2.442	2.12	2.76	2.442
$T_1R_1C_1$	0.97	1.36	1.17	1.08	1.21	1.15	1.32	1.10	1.21	1.61	1.18	1.40	1.33	1.10	1.22	1.69	1.09	1.39
C_3	1.02	1.38	1.20	1.56	1.07	1.32	1.20	1.01	1.11	1.67	1.21	1.44	1.17	1.04	1.11	1.54	1.08	1.31
C_6	1.05	1.28	1.17	1.52	1.20	1.36	1.32	0.93	1.13	1.81	1.07	1.44	1.25	0.85	1.05	1.35	0.96	1.16
C_7	1.19	1.36	1.28	1.54	1.97	1.26	1.40	0.94	1.17	1.63	0.79	1.21	0.60	0.84	0.72	1.08	0.91	1.00
R_3C_1	1.68	1.54	1.60	1.67	1.35	1.51	1.68	1.30	1.49	1.99	1.27	1.63	1.68	1.10	1.39	1.84	1.37	1.61
C_3	0.98	1.37	1.18	1.49	1.30	1.40	1.39	1.03	1.21	1.72	1.15	1.44	1.25	1.03	1.14	1.56	1.10	1.33
C_6	1.32	0.93	1.13	1.34	1.17	1.26	1.08	0.95	1.02	1.72	1.06	1.39	0.80	0.92	0.86	1.53	1.00	1.27
R_6C_1	1.24	1.36	1.30	1.65	1.20	1.43	1.13	1.01	1.07	1.43	1.31	1.37	1.14	1.01	1.08	1.56	1.11	1.34
C_3	1.12	1.42	1.27	1.53	1.18	1.36	1.41	0.93	1.17	1.50	1.18	1.34	1.16	0.99	1.06	1.58	1.08	1.33
R_7C_1	1.24	1.36	1.30	1.47	1.34	1.41	1.24	1.01	1.13	1.03	1.21	1.12	1.43	1.04	1.24	1.51	1.16	1.34
$T_3R_1C_1$	1.09	1.24	1.17	1.75	1.18	1.47	1.43	1.17	1.30	1.67	1.17	1.42	1.37	1.04	1.21	1.41	1.13	1.27
C_3	1.49	1.33	1.41	1.13	1.07	1.10	0.94	1.13	1.04	1.44	1.04	1.24	0.98	0.93	0.96	1.31	1.08	1.20
C_6	1.12	1.32	1.22	1.56	1.04	1.30	0.93	0.92	0.93	1.47	0.87	1.17	1.01	0.93	0.96	0.94	0.99	0.97
R_3C_1	1.26	1.16	1.21	1.43	1.23	1.33	1.13	1.18	1.16	1.34	1.17	1.26	1.17	1.01	1.09	1.79	1.21	1.45
C_3	1.16	1.38	1.27	1.62	1.01	1.32	1.48	1.14	1.31	1.29	1.09	1.19	1.25	1.03	1.14	1.32	1.06	1.19
R_6C_1	0.91	1.27	1.09	1.43	1.11	1.27	1.31	1.05	1.18	1.10	0.85	0.98	1.00	1.00	1.00	1.33	1.04	1.19
$T_5R_1C_1$	1.10	1.26	1.18	1.56	1.12	1.34	1.36	1.11	1.24	0.87	0.88	0.88	1.15	1.05	1.10	1.69	1.19	1.44
C_3	1.16	1.20	1.18	1.52	0.91	1.22	1.07	1.00	1.04	1.16	0.88	1.02	1.06	0.93	1.00	1.23	0.92	1.08
R_3C_1	1.00	1.15	1.08	1.59	1.12	1.36	1.45	1.18	1.32	0.93	0.95	0.94	0.88	0.88	0.88	1.29	0.85	1.07
$T_7R_1C_1$	1.04	1.04	1.04	1.05	0.97	1.01	1.26	1.04	1.15	0.94	0.44	0.69	0.80	0.83	0.82	1.20	0.80	1.00

corresponding value of 1.61. It will be observed that the graph representing yields for group V lies below the graph of the 3-salt group throughout its en-

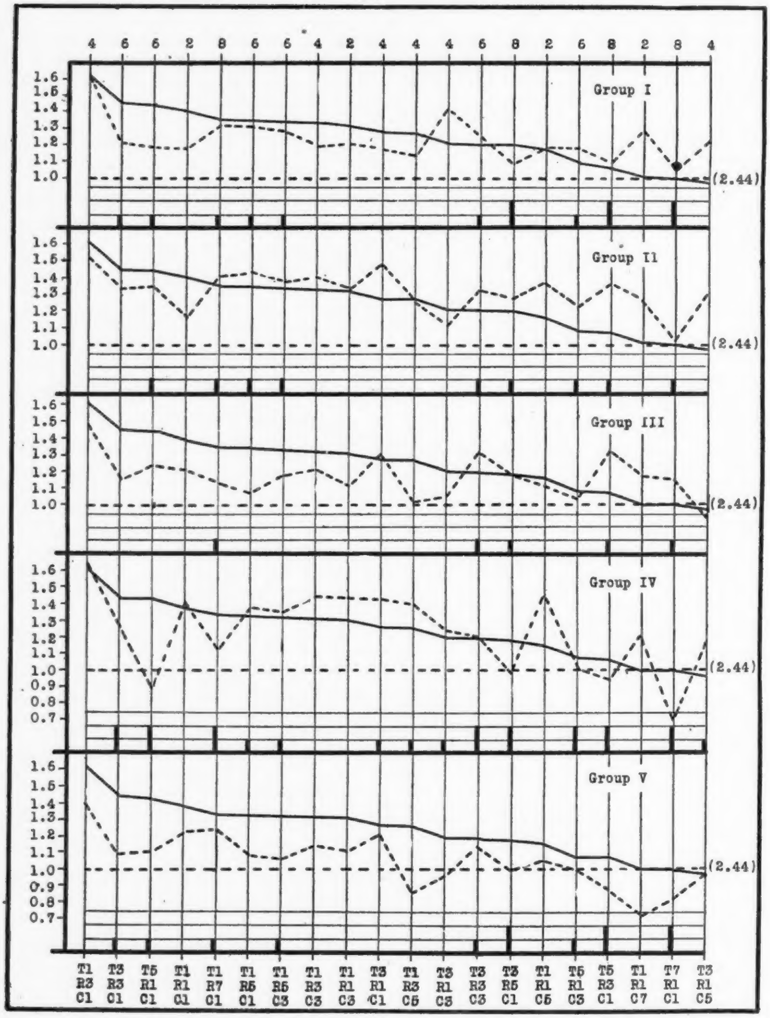

FIG. 2. RELATIVE DRY WEIGHTS AND INJURY OF SOYBEAN TOPS GROWN IN SOIL CULTURES CONTAINING PHOSPHATES IN CONNECTION WITH A COMPLETE FERTILIZER RATION

tire length. It is thus clear that each set of salt proportions of the 3-salt group is decreased in productiveness by the presence of mono-magnesium phosphate.

The graph representing the average yield from the 3-salt group shows clearly enough that the soil here employed was improved for the growth of soybeans by the application of all but three of the various sets of salt proportions of the three salts, KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, in solutions having total osmotic concentration values of 2.5 atmospheres, when the applications of the solutions were made to yield a soil moisture content of 12.5 per cent on the basis of the weight of the air-dry soil (500 cc. of solution to each 4 kgm. of soil). The maximum average yield of the 3-salt group was obtained from culture $T_1R_3C_1$. The yield from this culture showed an improvement of 61 per cent over that of the average yield from the check cultures, while the minimum average yield, obtained from culture $T_3R_1C_5$, was 3 per cent below the average yield from the checks. The salt proportions of the solutions added to these two cultures are: KH_2PO_4, 0.0173 m.; $Ca(NO_3)_2$, 0.0040 m.; $MgSO_4$, 0.0322 m., for the former, and KH_2PO_4, 0.0074 m.; $Ca(NO_3)_2$, 0.0257 m.; $MgSO_4$, 0.0083 m., for the latter. With other total concentrations of the solutions than the one here employed, it may be expected that the salt proportions required to produce maximum growth would be different from the ones here noted, since it has been pointed out by several authors (3, 14, 12, 8, 9, 1) that physiological balance of salt proportions in nutrient media is markedly dependent upon total concentration. It is thus clear that many degrees of beneficial effects and some injurious ones upon the growth of soybeans may be obtained by the application of various proportions of the three salts, when the total quantities of the three salts in solution applied to each soil culture, have approximately equal osmotic values. This emphasizes the importance of considering, not only salt proportions, but also total quantities applied (concentration), in the use of fertilizer salts in agricultural practice.

The graphs representing the yields of group I (NaH_2PO_4) and group II [$(NH_4)H_2PO_4$] show that all the average yields from the cultures of these groups were above the average yield from the check cultures, while groups III, IV, and V show one, four, and five cultures, respectively, which produced lower average yields than did the checks. As is thus clearly indicated, the soil employed in these cultures was improved for the growth of soybean tops by each set of salt proportions of groups I and II, and by all but one set of salt proportions of group III, while four sets of salt proportions of group IV, and five sets of group V, reduced the productiveness of this medium.

Further inspection of the graphs of figure 2 and also of the data of table 5 brings out the fact that in each of the five groups the highest average yield occurred with culture $T_1R_3C_1$, which corresponds also with the culture of the 3-salt group producing the highest yield. The maximum average yields for these five groups, relative to the average yields from the check cultures, are as follows: group I, 1.60; group II, 1.51; group III, 1.49; group IV, 1.63; group V, 1.39. The maximum yield from the 3-salt group has a corresponding value of 1.61, which is slightly surpassed only by the maximum average yield from group IV, having a value of 1.63.

2. Injury. The cultures of the 3-salt group suffered no specific injury, but remained in an apparently healthy and vigorous condition throughout the entire growth period. The cultures of the other five groups gave no evidence of disturbed growth during the first two weeks after the young plants had been placed in the soil cultures containing the monobasic phosphates in addition to the complete nutrient mixture. The plants showed differences in size, but no unusual phenomena occurred during this time. During the third week however, evidences of disturbed growth began to appear in the plants of several cultures of each group. The cotyledons were the parts first to be injured. The injury appeared in the form of dark brown discolorations around the margins of these organs. Later it appeared on the foliage leaves and in a few cases, on the stems of the plants also. In no culture, however, was the injury sufficiently severe to cause the death of the plants before the end of the growth period.

Careful comparisons revealed no differences between the characteristics of the injury sustained by the plants of the various groups. The injured cultures of these five groups were also compared with those of the preceding experiment, where the media in which the plants were rooted contained the monobasic phosphates singly. Aside from the varying degrees of injury, this comparison showed no apparent differences in the nature of the injury sustained by the cultures of the two experiments. It appears, therefore, that the nature of the toxin producing the specific injury here observed is the same, not only in the cultures of the groups employing the different monobasic phosphates singly, but also in those containing these phosphates in combination with the complete nutrient mixtures.

The injured cultures and the degrees of injury sustained are indicated in figure 2 by short, broad, vertical lines just below the graphs representing the relative yields, in a manner similar to that employed in figure 1. Vertical lines of two lengths only are employed in figure 2, since, as above stated, no severe injury was observed in the cultures of this experiment. The shorter vertical lines indicate slight injury, and the longer ones denote pronounced injury. As previously stated, the numbers at the top indicate the number of tenths of the total osmotic concentration (2.5 atmospheres), due to the total phosphates in the solutions whose numbers are given below.

The diagrams of figure 2 show that the number of injured cultures varied with the different groups as in the preceding experiment. The least total injury occurring in a single group is indicated for group III, with five cultures only slightly injured. The largest number of injured cultures exhibited by a single group occurred in group IV. This group comprised eight cultures which showed pronounced injury and six which were slightly injured, leaving only six uninjured cultures in the entire group. Groups I and V each comprised three cultures which showed pronounced injury and six with slight injury, making a total of nine injured cultures in each group, while group II exhibited the same number of injured cultures, none of which, however, showed pronounced injury.

It will be observed that in every group except group IV, the injury was confined to those cultures, each of which had received an application of solution whose partial osmotic concentration due to the total phosphates was not less than six-tenths of the total concentration of the solution, while pronounced injury occurred only in those cultures prepared with solutions having eight-tenths of their total concentrations due to the phosphates. In group IV, slight injury was observed in four cultures ($T_3R_1C_1$, $T_1R_3C_5$, $T_3R_1C_3$, and $T_3R_1C_5$) having four-tenths, and two cultures ($T_1R_5C_1$, and $T_1R_5C_3$) having six-tenths of their total osmotic concentrations due to the phosphates, while all the remaining cultures prepared with solutions having six-tenths or eight-tenths of their total concentration due to the phosphates, showed pronounced injury. It is thus clear that the $CaH_4(PO_4)_2$ used in the preparation of the cultures of this group has a higher capacity for producing this injury when used in connection with a complete nutrient mixture, than have any of the other phosphate salts here employed.

It is important to note that the cultures of groups II, III, and V, prepared with solutions having six-tenths of their total osmotic concentrations due to total phosphates, did not all suffer injury, nor did all the cultures prepared with solutions whose partial concentrations due to the phosphates were eight-tenths of the total osmotic concentration, sustain pronounced injury. Conditions similar to these prevailed also in group I, and in a more striking manner in group IV, where out of a total number of six cultures prepared with solutions having six-tenths of their total osmotic concentration due to the phosphates, four showed pronounced injury and two showed slight injury, and out of a total number of six cultures prepared with solutions, four-tenths of whose total concentrations were made up of the phosphates, four were slightly injured and two showed no injury at all. It is thus obvious that the degree of the injury sustained by the cultures of this experiment was not determined solely by the total phosphate content of the medium in which the plants were grown, but was largely influenced by the relative proportions of the four salts.

3. Relation of injury to yields. There appears to be no clearly definite relation between the injury here observed and the dry weight of tops, but in a general way pronounced injury is coincident with relatively low yields. Slight injury appears to have no greater tendency to occur with low yields than with high yields. For example, two cultures, $T_3R_3C_3$ and $T_5R_3C_1$, of group III each showed slight injury and gave relatively high yields of tops, while cultures $T_1R_3C_5$ and $T_3R_1C_3$ of the same group remained uninjured but produced relatively low yields. In group II, the cultures $T_1R_1C_1$ and $T_3R_1C_3$, both uninjured cultures, produced low yields, while the slightly injured cultures $T_1R_7C_1$ and $T_1R_5C_1$ each yielded relatively high dry weights of tops. Examples similar to these might be pointed out in each of the five groups. It is clear, therefore, that in the soil cultures here employed, slight injury does not materially interfere with the production of high yields of tops.

IV. Experiment III. Monobasic phosphate salts in solution cultures with complete nutrient mixtures

The solutions used in this experiment were the same as those used in the preparation of the soil cultures of the preceding experiment. The preparation of the solution cultures, the germination of the seeds, and the general treatment of the plants during the growth period have been described in a previous section. The formulas for the solutions of the five four-salt series are presented in table 2, and in a similar manner the composition of the solutions of the three-salt series is given in table 3. Each group thus comprised 20 cultures. An additional culture in Knop's solution with the same total osmotic concentration (2.5 atm.) was added to each group.

The experiment was carried out in the greenhouse with the cultures arranged in rows (one group of cultures in each row) on tables centrally located. In order to expose the cultures as nearly alike as possible to the constantly changing environment, they were regularly shifted in their positions according to a definite plan. Each of the two series of cultures here considered extended over a time period of 24 days. Series A was conducted from January 8 to February 1. Series B, which was a repetition of series A was carried out between February 7 and March 4, 1917.

1. Dry weights of tops. The dry weight yields of tops expressed in terms of the average yield from Knop's solution taken as 1.00, are presented in table 6. The data in this table are arranged in the same manner as are those in table 5. The actual average yield of tops from Knop's solution is given in parenthesis below the relative value (unity) in each case, so that the actual value of any culture may be calculated. It will be observed that the data for five groups only appear in the table. The data for group IV are here omitted, owing to the fact that the solutions containing $CaH_4(PO_4)_2$ proved entirely unsuited to the growth of soybeans. The plants of most of the cultures were so severely injured that growth was impossible, and the plants of all the cultures except those of cultures $T_1R_1C_1$, $T_1R_1C_3$, $T_1R_1C_5$, and $T_1R_1C_7$, were killed before the end of the growth period. The cultures of this group were not repeated.

As in the case of the yields from the soil cultures of the preceding experiment, the data of table 6 have been graphically represented and these graphs are presented in figure 3. In both cases the data for the 3-salt group of cultures were arranged in the decreasing order of their values and then plotted as ordinates, with the abscissas arbitrarily taken to represent the different cultures. The order of the cultures is thus not the same in figures 2 and 3. After the descending order of values for the 3-salt group was determined, the data for the 4-salt groups were plotted in this order, with the same scale of ordinates in each case, to form the four graphs represented by the irregular dotted lines. The graph representing the yields from the 3-salt group is superimposed upon each of the other graphs for ready comparison. The horizontal dotted line

TABLE 6

Relative dry weights of soybean tops grown 24 days in culture solutions, all with total concentrations having osmotic values of approximately 2.5 atmospheres. Series A, conducted from January 8 to February 1; Series B, from February 7 to March 3, 1917

CULTURE NUMBER	GROUP I			GROUP II			GROUP III			GROUP V			3-SALT GROUP		
	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average
Knop's solution	1.00 (0.70)	1.00 (0.66)	1.00 (0.679)	1.00 (0.70)	1.00 (0.66)	1.00 (0.679)	1.00 (0.70)	1.00 (0.66)	1.00 (0.679)	1.00 (0.70)	1.00 (0.66)	1.00 (0.679)	1.00 (0.70)	1.00 (0.66)	1.00 (0.679)
$T_1R_1C_1$	1.38	1.45	1.42	1.38	1.14	1.26	0.64	0.62	0.63	0.64	0.50	0.57	1.55	1.28	1.42
C_3	1.50	1.42	1.46	1.19	1.18	1.19	1.15	1.07	1.11	0.62	0.69	0.66	1.43	1.64	1.54
C_5	1.27	1.35	1.31	1.20	1.29	1.25	1.20	1.14	1.17	0.44	0.45	0.45	1.28	1.18	1.23
C_7	1.40	1.37	1.39	0.87	1.04	0.96	0.70	0.72	0.71	0.60	0.56	0.58	0.74	0.91	0.83
R_3C_1	0.60	0.50	0.55	0.45	0.46	0.46	0.40	0.50	0.45	0.40	0.34	0.37	0.44	0.43	0.44
C_3	0.60	0.55	0.58	0.45	0.50	0.48	0.56	0.45	0.51	0.41	0.40	0.41	0.47	0.56	0.52
C_5	0.53	0.55	0.54	0.47	0.55	0.51	0.52	0.50	0.51	0.44	0.42	0.43	0.43	0.52	0.48
R_5C_1	0.39	0.45	0.42	0.32	0.47	0.40	0.46	0.42	0.44	0.44	0.30	0.37	0.36	0.46	0.41
C_3	0.46	0.46	0.46	0.41	0.48	0.45	0.46	0.38	0.42	0.38	0.43	0.41	0.41	0.45	0.43
R_7C_1	0.36	0.32	0.34	0.36	0.37	0.37	0.40	0.41	0.41	0.30	0.34	0.32	0.37	0.38	0.38
$T_3R_1C_1$	1.37	1.46	1.42	1.41	1.45	1.43	0.50	0.46	0.48	0.51	0.43	0.47	1.05	0.98	0.02
C_3	1.16	1.34	1.25	1.32	1.13	1.23	0.53	0.48	0.51	0.44	0.40	0.42	0.86	0.70	0.78
C_5	1.02	1.28	1.15	0.88	1.01	0.95	0.58	0.50	0.54	0.39	0.36	0.38	0.63	0.71	0.67
R_3C_1	0.93	0.55	0.74	0.42	0.50	0.46	0.50	0.41	0.46	0.40	0.52	0.46	0.44	0.47	0.46
C_3	0.74	0.59	0.67	0.42	0.46	0.44	0.41	0.37	0.39	0.40	0.40	0.40	0.42	0.50	0.46
R_5C_1	0.37	0.44	0.41	0.38	0.44	0.41	0.43	0.47	0.45	0.38	0.32	0.35	0.37	0.43	0.40
$T_5R_1C_1$	0.97	1.51	1.24	0.53	1.00	0.77	0.38	0.40	0.39	0.55	0.43	0.49	0.67	0.59	0.63
C_3	0.76	1.37	1.07	0.87	0.96	0.92	0.44	0.42	0.43	0.53	0.41	0.47	0.46	0.51	0.49
R_3C_1	0.82	0.75	0.79	0.44	0.56	0.50	0.45	0.36	0.41	0.33	0.45	0.39	0.36	0.50	0.43
$T_7R_1C_1$	0.66	1.11	0.89	0.58	0.71	0.65	0.50	0.46	0.48	0.42	0.40	0.41	0.48	0.53	0.51

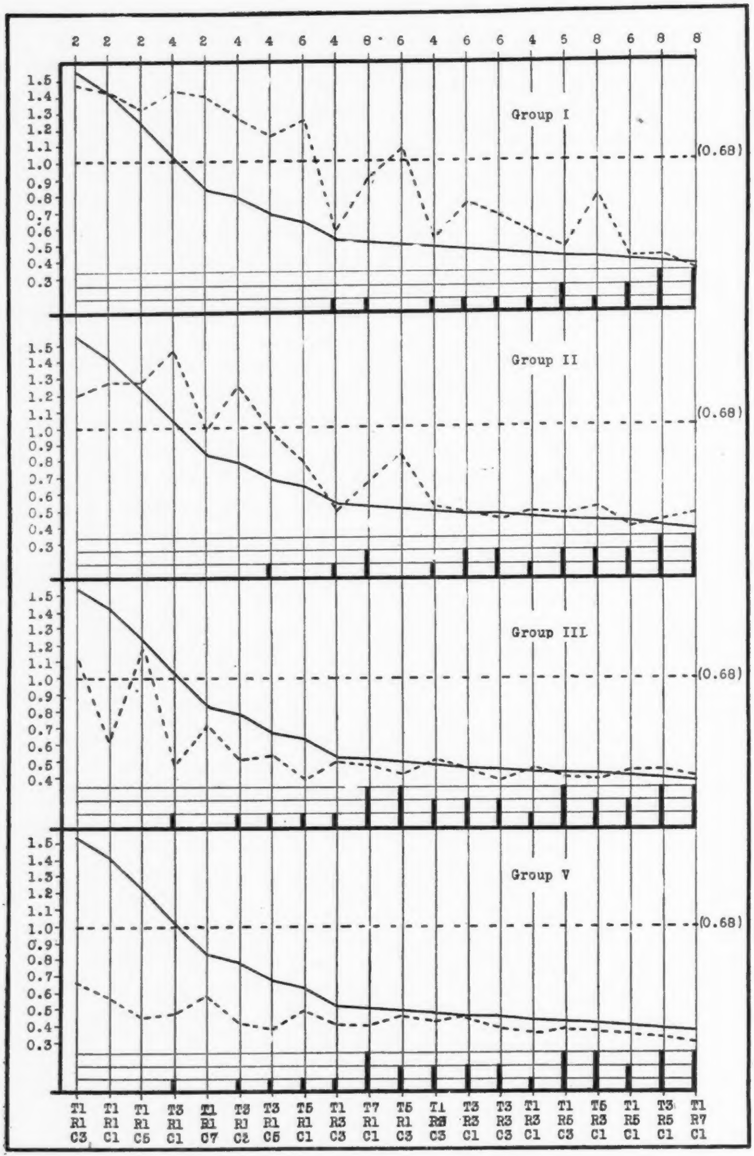

FIG. 3. RELATIVE DRY WEIGHTS AND INJURY OF SOYBEANS GROWN IN SOLUTIONS CONTAINING PHOSPHATES IN CONNECTION WITH COMPLETE NUTRIENT MIXTURES

here represents the average relative value of the yields from Knop's solution. The actual average value of these yields is given in parenthesis at the right. As in figure 2, the degrees of injury sustained by the cultures are represented by the broad perpendicular lines below each graph. The numbers at the top represent, in each case, tenths of the total osmotic concentration due to the total phosphate salts in the solutions whose numbers appear below.

Perhaps the most striking feature brought out by the graphs of figure 3 is the fact that all but two of the yields from the cultures of group I, and a l except five of the yields from group II, were markedly higher than were the yields from corresponding cultures of the 3-salt group, while the yields from all but five cultures of group III and all except one culture of Group V were lower than were the yields from corresponding cultures of the 3-salt group. The marked improvement of the yields from many of the cultures of Groups I and II, over the yields from corresponding cultures of the 3-salt group, may perhaps be explained by the fact that with the introduction of NaH_2PO_4 into the solutions of group I, and $NH_4H_2PO_4$ into the solutions of group II, a new ion was introduced into the solutions of each of these two groups: Na ions into the solutions of the former and NH_4 ions into the solutions of the latter. Here, possibly, the antagonistic effects of the new ions served to improve the media for the growth of soybeans in the general manner suggested by Osterhout (10). On the other hand, no new ions were added to the solutions of groups III and V with the introduction of the additional KH_2PO_4 into the solutions of the former and $MgH_4(PO_4)_2$ into those of the latter. The ions introduced into the solutions with these two salts served merely to increase the partial concentrations and to change the proportions of the ions given to the solutions by the three salts KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$.

It is important to note, however, that the maximum yield from the 3-salt group is not equaled by the maximum yield from any of the other groups. The highest yield from the 3-salt group occurred with culture $T_1R_1C_3$, having salt proportions as follows: KH_2PO_4, 0.0058 m.; $Ca(NO_3)_2$, 0.012 m.; and $MgSO_4$, 0.0323 m. The average yield from this culture had a dry-weight value of 1.54, relative to the average yield from Knop's solution taken as unity. This culture corresponded also to the culture ($T_1R_1C_3$) in group I and in group V producing maximum yields, while the highest yields in groups II and III occurred with cultures $T_3R_1C_1$ and $T_1R_1C_5$, respectively. The maximum average yields from groups I, II, III, and V were 1.46, 1.43, 1.17 and 0.66, respectively, relative to the average yield from Knop's solution.

2. Injury. While only the two cultures $T_1R_7C_1$ and $T_3R_5C_1$ of the 3-salt group were severely injured, the four cultures $T_1R_5C_1$, $T_5R_3C_1$, $T_1R_5C_3$ and $T_3R_3C_1$ showed pronounced injury, and the four cultures $T_1R_3C_1$, $T_3R_3C_3$, $T_7R_1C_1$ and $T_1R_3C_3$ sustained slight injury. The total injury sustained by the cultures of this group, however, was less than that of any of the other groups. The uninjured cultures made rapid and vigorous growth.

The interval of time which elapsed after the seedlings had been placed in

the solutions until the injury became apparent in the cotyledons of the seedlings, was very short. In two cultures ($T_1R_7C_1$ and $T_3R_5C_1$) of each group except the 3-salt group, the plants showed signs of specific injury on the second day after they had been placed in the solutions. All the cultures which remained uninjured at the end of 12 days suffered no injury at all during the remainder of the growth period, and some which had been only slightly injured completely recovered and made excellent growth. The time required for the appearance of the injury in the solution cultures here employed is thus in direct contrast with the time required for its appearance on the plants of the soil cultures of the preceding experiment, where no evidences of injury became apparent during the first two weeks of the growth period.

Careful comparisons were made between the injured cultures of the various groups in order to determine whether the characteristics of the injury differed in any respect with the cultures of the different groups, but no differences in the nature of the injury could be detected. Nor did comparisons of the plants in the water cultures of the present experiment with those of the soil cultures of the preceding experiments reveal any apparent difference in the nature of the injury to the cultures of the experiments here considered. It thus appears that the specific injury to the soybean plants of these experiments is directly related to some property which is common to the solutions employed in the preparation of all the cultures which show this injury.

Turning now to the diagrams of figure 3, representing the relative degrees of injury of the four groups, it will be observed that the number of injured cultures varies with the different groups, the smallest number of injured cultures being indicated for group I. This group comprises seven cultures which were slightly injured, two which showed pronounced injury, and two which suffered severe injury, making a total of eleven injured cultures for the entire group, leaving nine cultures which remained uninjured. Group II showed four cultures which sustained slight injury, six with pronounced injury, and two which suffered severe injury, leaving eight uninjured cultures in the group. Groups III and V each exhibited sixteen injured cultures, leaving only four uninjured cultures in each group. Six cultures of each of these two groups showed slight injury, five sustained pronounced injury, while five suffered severe injury. The number of uninjured cultures in groups III and V is thus seen to be only half the number in group II, and less than half the number in group I.

No injury was produced in any of the four groups by solutions whose partial concentration due to the total phosphate was not greater than two-tenths of the total concentration of the solution, although in groups III and V all the solutions with higher partial osmotic concentration of the phosphates produced the injury. In both group I and group II, however, the two solutions $T_3R_1C_1$, and $T_3R_1C_3$, with four-tenths, and solutions $T_5R_1C_1$ and $T_5R_1C_3$, with six-tenths of their total concentrations due to the phosphates, produced no injury.

In the four groups of cultures here considered, it is obvious that not only do the degrees of injury sustained by the plants vary with the variations in the partial osmotic concentrations of the total phosphates, but also with variations in the proportions of the four salts. This is well brought out in group I, where, out of a total number of six cultures with solutions having partial osmotic concentrations due to total phosphates, equal to six-tenths of the total concentrations of the solutions, two cultures showed pronounced injury, two sustained slight injury, and two remained uninjured. In the same group, out of a total number of four solutions with eight-tenths of their total osmotic concentrations due to the phosphates, two solutions, $T_3R_5C_1$ and $T_1R_7C_1$, produced severe injury, while the remaining two solutions, $T_7R_1C_1$ and $T_5R_3C_1$, produced only slight injury. Of the total number of seven solutions of this group which produced slight injury, two had eight-tenths, two had six-tenths, and three had four-tenths of their total osmotic concentrations due to the total phosphates in the solutions. Similar relations of injury to salt proportions and to partial osmotic concentrations due to the total phosphates in the solutions, are clearly indicated also in each of the groups II, III and V. This emphasizes the point already brought out in connection with a similar study of the corresponding groups of soil cultures of the preceding experiment, that the degree of injury sustained by the various cultures is not determined solely by the total phosphate content of the medium in which the plants are grown, but is related also to the proportions of the salts.

3. Injury to roots. Frequent inspection of the roots of the various cultures during the first few days of the growth period, revealed the fact that in those solutions which produced pronounced and severe injury to the tops, the roots were checked in their development soon after being placed in the solutions, and showed specific injury even before disturbed growth in the tops became apparent. At the end of the first week after the seedlings had been placed in the solutions, the injured root systems were characterized by a general unhealthy appearance and by the suppression of lateral root development. Microscopical examination at the end of a week's growth showed the root tips of the more severely injured cultures to be dead, and some of the cells of the root tips to be in a state of disintegration. The roots of the cultures thus affected gradually turned brown in color and the roots of the two cultures $T_1R_7C_1$ and $T_3R_5C_1$ of each group, with the roots of cultures $T_7R_1C_1$, $T_5R_1C_3$, and $T_1R_5C_3$ from group III, and those of cultures $T_7R_1C_1$, $T_1R_5C_3$, and $T_5R_3C_1$ from group V, were killed before the end of the growth period. This latter condition of the roots was always coincident with severe injury to the tops. In a few of the cultures ($T_7R_1C_1$, $T_3R_3C_1$, and $T_5R_3C_1$ of group I, and $T_3R_1C_5$ of group II), which were characterized by slight leaf injury, the roots appeared to be especially well developed, showing evidences of stimulated root growth, while most of the cultures thus characterized showed root development which was somewhat below the average. In cultures where no leaf injury at all was apparent, root development was excellent, but not quite so vigorous as in those cultures above

indicated which suffered slight injury but also gave evidence of stimulated root growth.

4. Relation of injury to yields. In order to facilitate the study of the relations between the injury here observed and the average relative yields of tops, reference may be had to the graphs and diagrams of figure 3. It is to be observed that the lowest average yields occurred with those cultures which suffered either pronounced or severe injury, while the highest average yields were obtained from the cultures which were free from injury. This relation is clearly definite for each group of cultures. In general it may be said that those cultures of each group which suffered slight injury produced relatively low yields, while medium yields were obtained from cultures without injury.

Variations in the yield values with variations in the degrees of the injury sustained, were comparatively slight, since all the cultures which suffered severe, pronounced or slight injury produced relatively low yields. In these cultures, therefore, the injury appears to be the controlling factor in the production of low yields. This is in direct contrast to the conditions which prevailed in this regard, in the corresponding soil cultures of the preceding experiment, where some of the slightly injured cultures produced relatively high yields and the cultures which suffered pronounced injury gave low yields.

That many of the cultures of group I and group II produced markedly higher yields (relative to the yields from the cultures of the 3-salt group) than did the corresponding cultures of groups III and V, has already been pointed out in connection with a consideration of the dry-weight values. This marked increase in yields was accompanied by a corresponding decrease in the injury sustained by the cultures, as is clearly indicated by the graphs and diagrams of figure 3. Here also it is possible that the antagonistic effects of the new ions, Na and NH_4, introduced into the solutions of group I and group II with NaH_2PO_4 and $(NH_4)H_2PO_4$, respectively, may be responsible for the decrease in the injury produced as well as for the marked improvement in the yields.

5. Relation of acidity to yield and to injury. The H-ion concentration is a characteristic of the culture solutions which may be expected to bear some relation to the yields produced and to the injury sustained by the cultures. It has already been pointed out that the injury sustained by the soybean plants is related to the atomic group H_2PO_4 or to the ions resulting from the partial dissociation of this group in the solutions. The necessary apparatus to determine the H-ion concentration of these solutions electrometrically was not at hand. An attempt was made, however, to determine the acidity of the solutions whose formulas are given in tables 2 and 3, by titration methods, and to relate the results thus obtained to the yield of tops and to the injury sustained by the plants.

In making the acidity determinations, 100 cc. of the solution to be tested was diluted with 150 cc. of distilled water, and was then titrated with $\frac{N}{10}$ NaOH, using as indicators alizarin and methyl orange. Several tests of all the solutions were made with each of these indicators. The results of these tests are

included in table 7, which gives the total acidity (gram-molecules per liter) as here determined, in terms of phosphoric acid. Since the tests made with alizarin yielded results which were in very close agreement with those obtained by the use of methyl orange as an indicator, the results of all the tests for each solution were averaged. The values in the table represent, therefore, the average results of all the tests with each solution.

In order to bring out the relation between the acidity of the solutions and the yields of tops, and also the relation between acidity and injury, the graphs and diagrams of figure 4 were prepared. The dotted line in each set of graphs

TABLE 7

Acidity of culture solutions in terms of phosphoric acid

SOLUTION NUMBER	GRAM MOLECULES PER LITER				
	Series I	Series II	Series III	Series V	3-salt series
$T_1R_1C_1$	0.000199	0.000050	0.000149	0.000497	0.000000
C_3	0.000189	0.000050	0.000149	0.000546	0.000000
C_5	0.000149	0.000050	0.000099	0.000596	0.000000
C_7	0.000174	0.000050	0.000099	0.000546	0.000000
R_3C_1	0.000447	0.000224	0.000274	0.000720	0.000323
C_3	0.000348	0.000199	0.000224	0.000671	0.000298
C_5	0.000398	0.000199	0.000248	0.000546	0.000199
R_5C_1	0.000497	0.000447	0.000472	0.000918	0.000397
C_3	0.000475	0.000447	0.000472	0.000820	0.000397
R_7C_1	0.000621	0.000497	0.000497	0.000992	0.000695
$T_3R_1C_1$	0.000398	0.000199	0.000174	0.001290	0.000050
C_3	0.000298	0.000199	0.000174	0.001240	0.000050
C_5	0.000224	0.000149	0.000174	0.001440	0.000050
R_3C_1	0.000546	0.000348	0.000323	0.001890	0.000397
C_3	0.000447	0.000199	0.000274	0.001740	0.000348
R_5C_1	0.000646	0.000447	0.000447	0.001990	0.000645
$T_5R_1C_1$	0.000646	0.000447	0.000274	0.002640	0.000125
C_3	0.000447	0.000348	0.000224	0.002680	0.000075
R_3C_1	0.000696	0.000645	0.000472	0.003140	0.000497
$T_7R_1C_1$	0.000746	0.000423	0.000497	0.003680	0.000298

represents the actual average yield values and the unbroken line represents the acidity of the solutions (gram-molecules per liter) in terms of phosphoric acid. The values represented by the graphs for each group are here arranged in the descending order of the actual dry-weight values for the 3-salt group. The order of arrangement is indicated by the culture or solution numbers placed below, and is the same as that employed in the preparation of the graphs of figure 3. The relative degrees of injury are again indicated by broad vertical lines, in a manner similar to that employed in the preceding figures.

Inspection of the data of table 7 and the graphs of figure 4, brings out the

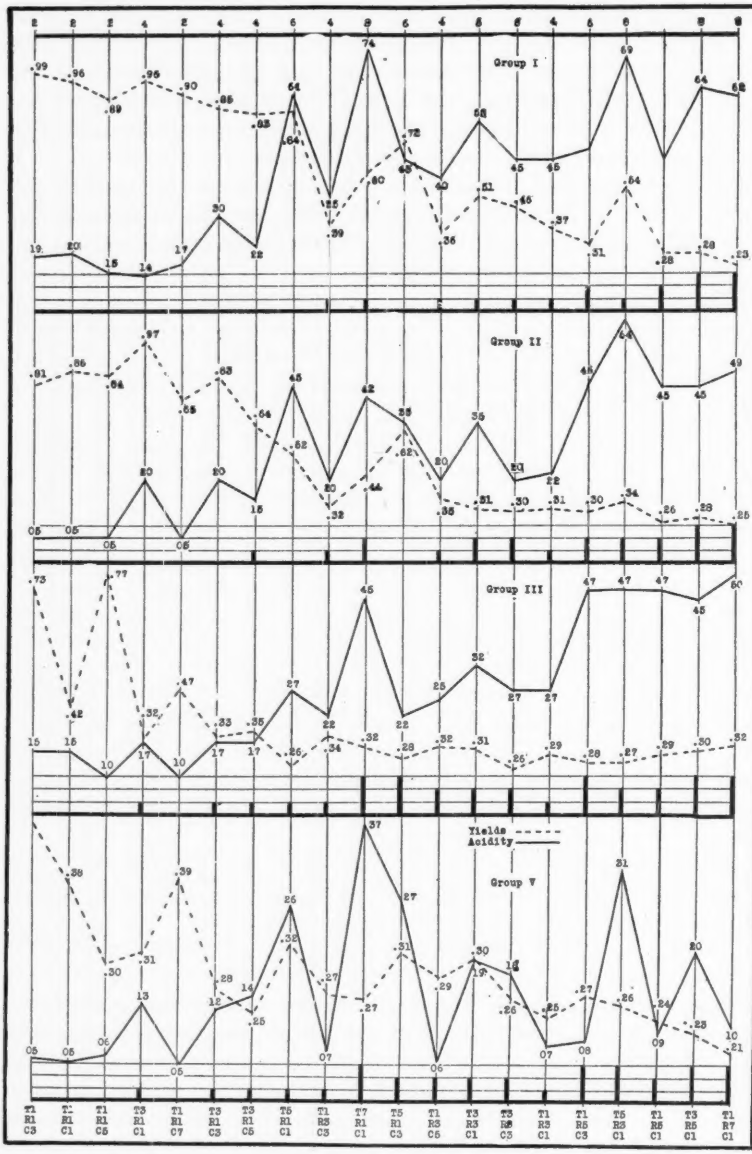

FIG. 4. RELATION OF ACIDITY TO YIELDS AND INJURY: GROUPS I–V. (ACIDITY VALUES ARE INDICATED ON CHART BY OMITTING PREFIX 0.000 FOR GROUPS I, II, AND III, AND 0.00 FOR GROUP V)

fact, as is to be expected, that with the increase in the partial osmotic concentrations of the total phosphates in the solutions, the acidity of the solutions is increased. The increase in acidity, however, is not proportional to the increase in concentration due to phosphates; the graphs, therefore, are somewhat irregular. While the acid content of any one of these solutions is mainly determined by the partial osmotic concentration of the total phosphates, it is modified by the relative proportions of the two phosphates (KH_2PO_4 alone in the solutions of group III) in the solution, and also by the relative proportions of the two constituent salts $Ca(NO_3)_2$ and $MgSO_4$.

It will be observed that while the graphs of figure 4, representing acidity, are very irregular, each graph shows a decided tendency to slope upward to the right. On the other hand, all the graphs representing actual average yields slope downward to the right. This clearly indicates a general tendency for the yields to be low in solutions having high acid content, and high in solutions which have low acid content. It is evident, however, that the relative salt proportions determine the kind of growth (good, medium or poor) which will take place in the solutions with medium or low acid content, since both high and low yields of tops occur with each of the four groups in the solutions thus characterized. When the acid content of the solution becomes excessive, the salt proportions appear to have little influence in determining the kind of growth, since only low yields are produced by the solutions with relatively high acid content. Two exceptions to the last statement are to be noted in cultures $T_5R_1C_1$ and $T_7R_1C_1$ of group I, each of which is high in acid, the former producing a relatively high yield and the latter a medium yield.

The graphs and diagrams of figure 4 indicate clearly that the solutions of each of the four groups here considered which produced no injury, were characterized by having low acidity as compared with that of the solutions which produced injury. Two exceptions to this appear in the case of the two solutions $T_5R_1C_1$ and $T_5R_1C_3$ of each of the groups I and II. Each of these solutions showed relatively high acidity but produced no injury. In group III the four solutions, $T_1R_1C_1$, $T_1R_1C_3$, $T_1R_1C_5$ and $T_1R_1C_7$, which produced no injury, were each characterized by having a lower acid content than any of the solutions of the same group which produced the injury. The uninjured cultures of group V correspond to those of group III, and the solutions of each of these cultures likewise were characterized by having a lower acid content than any solution of the same group which produced injury.

There is a general tendency in each of the four groups here considered for the injury to become more pronounced as the acid content of the solutions increases. This relation is, however, not absolutely definite. It is quite obvious that the relative salt proportions play a very important rôle in either accentuating or diminishing the injury when the acid content of the solutions is sufficiently high to produce the injury. One of two solutions having the same acid content and equal osmotic concentrations, may possess relative salt proportions which are favorable to good growth, while the relative salt propor-

tions of the other may not be well adapted to the growth of the plants. The salt proportions of the former would naturally tend to diminish the injurious effects of the acid, while the tendency of the latter would be to augment the injury. For example, the two solutions, $T_5R_1C_1$ and $T_3R_5C_1$ of group I, possessed the same acid content (0.000646) as this was here determined. The former produced a high yield and suffered no injury, while the latter gave a very low yield and sustained severe jnjury. In group II the four solutions $T_5R_1C_1$, $T_1R_5C_3$, $T_1R_5C_1$ and $T_3R_5C_1$, all had the same acidity (0.000447). The first of these solutions produced a medium yield but suffered no injury, while of the remaining solutions, the first two suffered pronounced injury, the last sustained severe injury, and all three gave very poor yields.

The relation between the injury sustained by the plants and the acid content of the solutions of the 3-salt group, is quite definite, as will appear from a study of figure 5. The data of the 3-salt group are here graphically presented

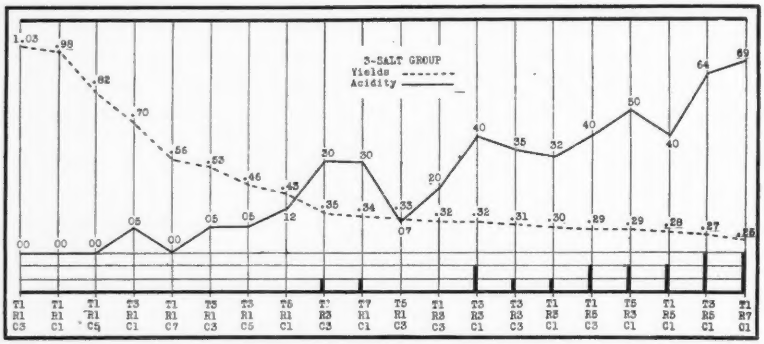

FIG. 5. RELATION OF ACIDITY TO YIELDS AND INJURY: 3-SALT GROUP. (ACIDITY VALUES ARE INDICATED ON CHART BY OMITTING PREFIX 0.000)

in the same manner as are those of the four groups represented in figure 4. It will be observed that each culture of this group with a solution having an acid content higher than 0.000497 showed severe injury. Each culture whose solution had an acidity value of 0.000397 or 0.000497 showed pronounced injury, and each solution with an acid content of 0.000298, 0.000323 or 0.000348 produced slight injury. No injury was produced by any solution of the 3-salt group with an acidity value below 0.000298. A clear relation between acidity and injury is here indicated; the higher the acid content of the solutions above a certain value (0.000298), the greater is the degree of injury sustained by the plants. This relation is definite for the conditions of the present study and for the group of cultures here considered. Hoagland (6) found a somewhat similar relation between the growth of barley seedlings and the H-ion concentration of the medium. An acid condition of the medium was found to be favorable to the growth of the seedlings, and produced no injury when the

acidity was not in excess of a certain limit of the H-ion concentration. When, however, the H-ion concentration was considerably increased beyond the limit set for favorable growth, very decided injury was caused.

It is evident that the injury sustained by the plants of these tests is the result, not of one factor alone, but of combinations of factors. The complexity of the whole problem is such as to render the drawing of definite conclusions extremely difficult. It should be emphasized in this connection that the conclusions here reached with regard to the toxic influence of the various phosphates and salt combinations employed, must be understood to apply for no other set of experimental conditions than those under which these cultures were conducted, and in connection with soybean plants during the early stages of their growth.

SUMMARY

The experiments above presented deal with the influence of five different monobasic phosphates on the growth of soybeans, with special reference to the toxic symptoms produced under various experimental conditions. The tests involved three distinct sets of experiments. (a) The plants were grown in soil cultures to which the salts were added singly in solutions having osmotic concentration values varying from 0.5 to 7 atmospheres. Five groups of cultures corresponding to the five phosphates were employed. Each group comprised 11 cultures including one check culture without salts. (b) The plants were grown in soil cultures to which the phosphates were applied in connection with a complete fertilizer ration in solutions with varying salt proportions but with approximately constant total osmotic concentration values of 2.5 atmospheres. Six groups of cultures (including one control group which employed only the three salts KH_2PO_3, $Ca(NO_3)_2$ and $MgSO_4$) each comprising 20 different sets of salt proportions, were employed. (c) The mixed solutions employed in the preparation of the soil cultures were used also for water cultures, without alteration. A trace of iron as ferric phosphate was added to each solution. For each culture 500 cc. of solution were used.

The total salts added to the soil cultures were included in a single initial application. Each soil culture comprised five plants. With water cultures, the solutions were renewed every third or fourth day and each culture comprised three plants.

The main results of these tests may be summarized as follows:

1. As indicated by relative yields, the growth of soybean tops was injuriously affected by each of the five phosphates employed singly in soil cultures, the average yield from the check cultures being considerably higher than the highest average yield from any of the treated cultures.

2. Each of the phosphates used singly in soil cultures caused specific injury to the plants when the solutions applied to the soil had osmotic concentration values above 1 atmosphere.

3. With respect to their capacities for producing specific injury, the phos-

phates employed singly in the soil cultures arrange themselves in the following order, beginning with the salt which is least toxic: (1) KH_2PO_4, (2) $(NH_4)H_2PO_4$, (3) $MgH_4(PO_4)_2$, (4) NaH_2PO_4, (5) $CaH_4(PO_4)_2$.

4. The nature of the injury produced by the five phosphates is identical. The injury is related to the common group H_2PO_4, or to the ions resulting from the dissociation of this group in the soil solution.

5. The five groups of soil cultures each employing a single phosphate in connection with the three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, produced their average maximum yields in corresponding cultures. The phosphates employed arrange themselves in the following order with respect to the production of maximum yield values, beginning with the highest: (1) $CaH_4(PO_4)_2$, (2) NaH_2PO_4, (3) $(NH_4)H_2PO_4$, (4) KH_2PO_4, and (5) $MgH_4(PO_4)_2$. The maximum yield from the 3-salt group was slightly surpassed only by that from the group employing $CaH_4(PO_4)_2$.

6. The plants of a number of soil cultures from each of the five groups employing a phosphate in connection with a complete fertilizer ration, suffered pronounced injury but with no culture was the injury sufficiently severe to cause the death of the plants before the end of the growth period. With respect to their capacity for producing the injury, the phosphates arrange themselves in the same order as they do when employed singly in soil cultures.

7. No specific injury occurred to the plants in any soil culture of the 3-salt group.

8. In each group of soil cultures except the one employing $CaH_4(PO_4)_2$, specific injury was confined to the cultures prepared with solutions whose partial osmotic concentration values due to total phosphates was not less than six-tenths of the total osmotic concentration. In the group employing $CaH_4(PO_4)_2$, injury was observed in cultures prepared with solutions having four-tenths of their total osmotic concentrations due to total phosphates.

9. The degree of injury sustained by the plants was not determined solely by the total phosphate content of the medium, but was related also to the relative proportions of the constituent salts.

10. With these soil cultures there was no definite relation between injury and yields. In general, pronounced injury was coincident with relatively low yields. Slight injury had no greater tendency to occur with low yields than with high yields.

11. In water cultures, the solutions employing NaH_2PO_4 and those employing $(NH_4)H_2PO_4$ in connection with the three salts KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, produced markedly higher yields with nearly every set of salt proportions, than did the corresponding solutions similarly employing KH_2PO_4 or $MgH_4(PO_4)_2$. This is attributed to the antagonistic effects of the new ions Na and NH_4 introduced into the solutions with NaH_2PO_4 and $(NH_4)H_2PO_4$, respectively.

12. The various phosphates used in water cultures with the 3-salt nutrient solutions (neglecting $CaH_4(PO_4)_2$, which rendered the solutions extremely toxic

to the soybean plants, with each set of salt proportions) arrange themselves in the same order, with respect to the production of maximum yield values, as they do in the corresponding groups of soil cultures. The average maximum yield from the 3-salt group was not equaled by that from any other group of solution cultures.

13. The specific injury sustained by the plants in solution cultures was more pronounced than that sustained by the plants in the corresponding soil cultures. With respect to their relative toxicities when employed in solution cultures with complete nutrient mixtures, the phosphates arrange themselves in the following order, beginning with the salt which is least toxic: (1) NaH_2PO_4, (2) $(NH_4)H_2PO_4$, (3) KH_2PO_4, (4) $MgH_4(PO_4)_2$, and (5) $CaH_4(PO_4)_2$.

14. Careful comparisons of injured plants from all the different media here considered showed the nature of the specific injury sustained by the plants to be identical.

15. Neglecting the solutions employing $CaH_4(PO_4)_2$, no injury was sustained by the plants grown in solutions whose partial osmotic concentrations due to total phosphates was less than four-tenths of the total osmotic concentration.

15. The relation between yields and injury with the solution cultures may be expressed as follows: The greater the degree of injury (considering only injured cultures) the lower the yields. High yields were obtained from solutions which produced no injury.

17. The acid content of the solutions bears a definite relation to the specific injury sustained by the plants grown in them: the higher the acid content (as here determined by titration methods) the greater the degree of injury suffered by the plants. The relative salt proportions, however, play a very important rôle in either accentuating or diminishing the injury when the acidity of the solutions is sufficiently high to produce it.

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EFFECT OF AMMONIUM SULFATE IN NUTRIENT SOLUTION ON THE GROWTH OF SOYBEANS IN SAND CULTURES

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INTRODUCTION

Since the middle of the last century a great amount of work has been done in furthering our knowledge on the question of salt requirements for green plants. Perhaps the most logical method of determining the best salt proportions for the growth of plants in water cultures was that recently adopted by Tottingham (29). Using Knop's (13) formula as a basis, Tottingham varied the proportions of the component salts by the increments of one-tenth of the total osmotic concentrations. All possible salt proportions were thus included in his series, necessitating the use of 84 different solutions. Tottingham was thus able to develop a nutrient solution for wheat in its early stage of growth that produced yields 11 per cent higher than did Knop's solution with the same total concentration (osmotic value 2.5 atm.).

Tottingham's solution contains four salts, KH_2PO_4, KNO_3, $Ca(NO_3)_2$ and $MgSO_4$. It will be observed that KNO_3 may be omitted from the Tottingham's solution without omitting any ion which is not furnished by the remaining salts. Thus, by using Tottingham's general method, Shive (26) has found that the omission of KNO_3 from the nutrient solution employed by Tottingham (29) not only did not cause any ill effect on the growth of young wheat plants, but actually produced an increased yield over that from Tottingham's best solution with the same total concentration (osmotic value 1.75 atm.).

This behavior of the 3-salt solution, on the other hand, suggests another possibility. Might not some other salt, although not containing any element that is not already present in the nutrient solution, benefit plant growth if added in proper proportion. Such a salt might be ammonium sulfate. Adding no new elements, it would introduce a new ion that could have some influence on the plant growth when present in different proportions with other ions in the nutrient solution.

It has been demonstrated that some plants can use ammoniacal nitrogen in the absence of NO_3. Gerlach and Vogel (6) grew corn seedlings in sterilized soil with the solution devoid of any nitrates, but containing ammonium sulfate along with the other elements required for plant growth, and found that a considerable amount of nitrogen was absorbed by the plants as compared with the amounts absorbed from untreated soil. Prianishnikov (24) classified the

most important field crops into three groups. The plants of the first group, including corn, barley, pumpkin, etc., can be supported readily by ammonium chloride or ammonium sulfate as a source of nitrogen. The second group (peas, vetches, etc.) suffered injury when treated with ammoniacal nitrogen, while the third group, comprising a single plant, *Lupinis luteus*, can not make a normal growth when treated with this kind of nitrogenous compounds. In connection with the latter plant reference can be made to the work of Nikolaeva (21). Therefore, it is possible that ammonium sulfate may have a nutritive effect in a nutrient solution that may result in the better development of the plants.

It is a well known fact that a cation of any salt, if this salt is present alone in the medium, has a toxic effect on living organisms, while no such injury is observed, if the properly balanced mixture of two or more salts is present in solution. For a very extensive review of literature on this important and interesting subject, reference may be made to McCool's (20) work and also to the exhaustive monograph of Frear (5). Osterhout (22) points out that the injurious effect of the NH_4-ion on plant growth could be antagonized by one of the following ions: Ca, Na and K. McCool's extensive experiments strongly support this contention of Osterhout. Working mainly with chlorides McCool (20) has found that the toxic action of NH_4Cl upon Canada field peas could be overcome, at least in part, by either Ca or Na in the chloride form.

The action of NH_4 in the form of sulfates on the plant tissue, perhaps, differs considerably from that of the same ion in a chloride, but the two cases could well be expected to be in parallel.

In view of the foregoing facts, it was deemed desirable to test this salt with Tottingham's nutrient solution, using the salt to be tested in the place of KNO_3. With our knowledge of the behavior of Tottingham's solution as compared with Shive's (26), an experiment of this nature would throw some light on the effect of ammonium sulfate when added to Shive's nutrient solution.

Two chief methods are employed by plant physiologists in most of the partially controlled experiments, namely, water culture and sand culture methods. Of the two, the sand culture, as a medium, more nearly approaches the natural conditions for plant development, as these conditions exist in soil. This method was chosen for the present work.

EXPERIMENTAL

Method employed

The experimental work herein recorded was done with soybeans of the "Black Eyebrow" variety. The seed came originally from the United States Department of Agriculture and was used on experimental plots at the New Jersey Agricultural Experiment Station.

The plants were grown in sand cultures in earthenware glazed cylindrical

pots that are 12.5 cm. in height having an inside diameter of 10 cm. Fourteen hundred grams of sand, washed in distilled water and dried, was placed in each pot within about 1 cm. of the top. The water-holding capacity of the sand used was 23.40 per cent (the average of two determinations). The moisture content of the medium in which the plants are grown has considerable influence on the plant development, as is well shown by a number of investigators. In this connection the works of Hellriegel (11), Prianishnikov (23), Harris (9), and Tulaikoff (30) are noteworthy. Both Hellriegel and Tulaikoff have found that the best crop yield was obtained with the moisture content of the medium equivalent to about 60 per cent of the water-holding capacity of the soil. In view of these facts it was decided to maintain a proportionally

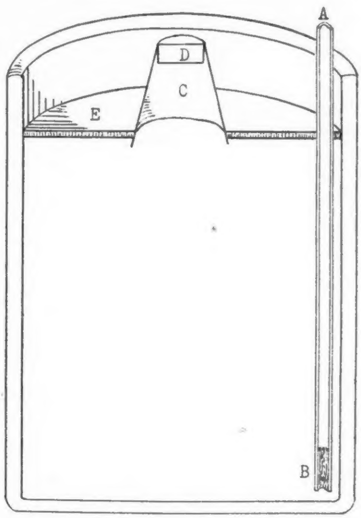

FIG. 1. DIAGRAM OF POT USED IN EXPERIMENTS
(Drawing by Dr. J. W. Shive)

similar moisture content in the sand cultures used in these experiments, which corresponded to 14 per cent of water based on the dry weight of sand in question.

The water content in the cultures was kept as constant as was practicable by adding water to the pots from day to day. For this purpose the pots were weighed every other day, but water was added daily in amount corresponding to its loss during the previous day. Every three or four days the solution in the sand cultures was changed according to a method similar to that employed by McCall (19), the difference being in the design of the pots. Instead of drawing off the solution at the bottom, as was done by McCall, provision was made to withdraw the excess solution at the top by means of a glass tube, which extended to the bottom of the pot. A glance at figure 1 reveals the main

features of the pot used in these experiments. The glass tubing may be of any convenient size and should be somewhat longer than the height of the pot. The lower portion was filled with glass wool to serve as a filter for the fine particles of sand. A small rubber tube was attached to the upper end

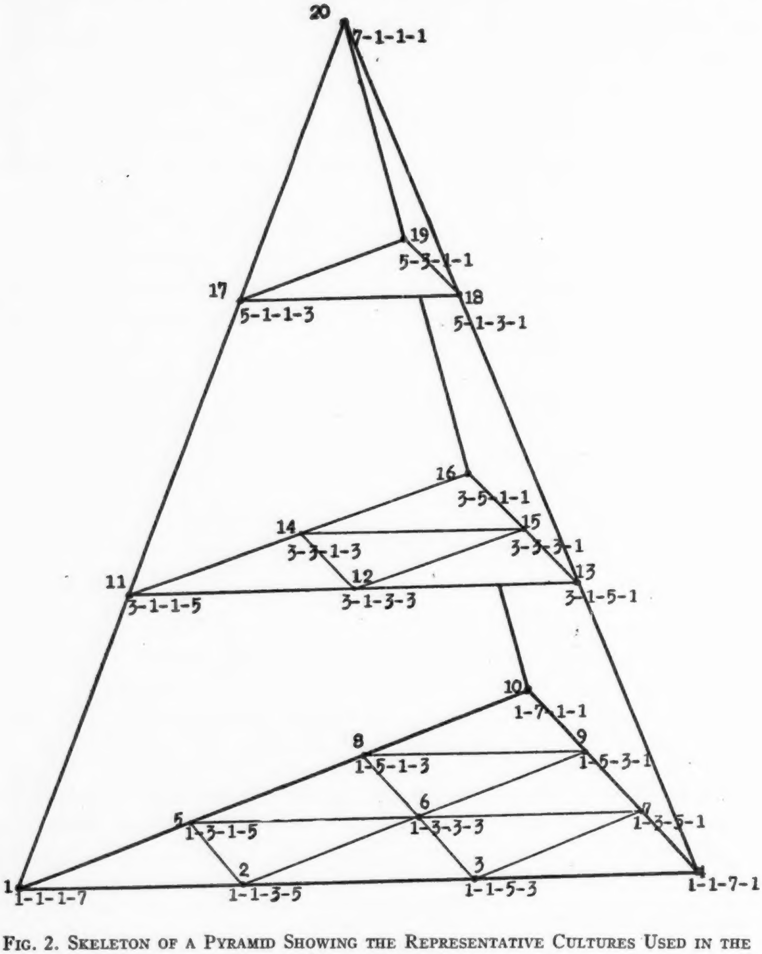

FIG. 2. SKELETON OF A PYRAMID SHOWING THE REPRESENTATIVE CULTURES USED IN THE PRESENT WORK

of the glass tube and thus served for connecting the pot with the suction outfit, which had a graduated cylinder to catch the solution. The changing of the solution was effected in the same manner as described by McCall (19). After planting the seedlings the surface of the sand in the pots was sealed

with a mixture of parowax and vaseline prepared according to the formula of Briggs and Schantz. The water and solution were added through the paper funnel. The paper funnels used in these experiments were prepared by rolling the manilla paper to form a cone, which was dipped into melted paraffine and then cooled. This was done to make it hold together. It was then cut to the desired length, inserted into the sand medium about 2 cm. deep in the middle of the pot, and corked. The length of the funnel used was 6 to 7 cm.

TABLE 1

Osmotic requirements of cultures

The figures in the table represent the number of cubic centimeters of the given salts of m/4 concentrations needed, when mixed together, and diluted to 250 cc., to make the concentration of resultant solution equal to approximately 2.5 atmospheres of pressure

POSITION OF CULTURES IN THE TRIANGLES OF THE PYRAMID	TOTTINGHAM SERIES					AMMONIUM SULFATE SERIES				
	Number of cultures	KH_2PO_4 m/4	KNO_3 m/4	$Ca(NO_3)_2$ m/4	$MgSO_4$ m/4	Number of cultures	KH_2PO_4 m/4	$(NH_4)_2SO_4$ m/4	$Ca(NO_3)_2$ m/4	$MgSO_4$ m/4
$T_1R_1C_1$	1	5.2	4.9	3.6	40.6	21	5.2	3.6	3.6	40.6
C_3	2	5.2	4.9	10.8	29.0	22	5.2	3.6	10.8	29.0
C_5	3	5.2	4.9	18.0	17.4	23	5.2	3.6	18.0	11.6
C_7	4	5.2	4.9	25.2	5.8	24	5.2	3.6	25.2	5.8
R_3C_1	5	5.2	14.6	3.6	29.0	25	5.2	10.8	3.6	29.0
C_3	6	5.2	14.6	10.8	17.4	26	5.2	10.8	10.8	17.4
C_5	7	5.2	14.6	18.0	5.8	27	5.2	10.8	18.0	5.8
R_5C_1	8	5.2	24.4	3.6	17.4	28	5.2	18.0	3.6	17.4
C_3	9	5.2	24.4	10.8	5.8	29	5.2	18.0	10.8	5.8
R_7C_1	10	5.2	34.2	3.6	5.8	30	5.2	25.2	3.6	5.8
$T_3R_1C_1$	11	15.6	4.9	3.6	29.0	31	15.6	3.6	3.6	29.0
C_3	12	15.6	4.9	10.8	17.4	32	15.6	3.6	10.8	17.4
C_5	13	15.6	4.9	18.0	5.8	33	15.6	3.6	18.0	5.8
R_3C_1	14	15.6	14.6	3.6	17.4	34	15.6	10.8	3.6	17.4
C_3	15	15.6	14.6	10.8	5.8	35	15.6	10.8	10.8	5.8
R_5C_1	16	15.6	24.4	3.6	5.8	36	15.6	18.0	3.6	5.8
$T_5R_1C_1$	17	26.0	4.9	3.6	17.4	37	26.0	3.6	3.6	17.4
C_3	18	26.0	4.9	10.8	5.8	38	26.0	3.6	10.8	5.8
R_3C_1	19	26.0	14.6	3.6	5.8	39	26.0	10.8	3.6	5.8
$T_7R_1C_1$	20	36.4	4.9	3.6	5.8	40	36.4	3.6	3.6	5.8

Solutions used

The solutions employed in this work were the same as in the work of Tottingham (29), who varied the osmotic concentration of his salt solution by one-tenth of their total osmotic concentration. The entire set of 84 cultures, however, was not used, but only 20 of the most representative cultures were selected, the selection being done in the following manner. The second, fourth and sixth triangles were entirely omitted. In the remaining first, third and fifth triangles every other row was omitted, and every other culture was

omitted in the remaining rows. This arrangement leaves 19 cultures in the three mentioned triangles. The twentieth culture used corresponded to the apex of the pyramid. Figure 2 gives a general idea of the place in the pyramid of the cultures employed. It will be seen to be a fairly representative skeleton. It also considerably lessens the work.

In parallel with the Tottingham cultures, another series of cultures was used, in which, instead of KH_2PO_4, KNO_3, $Ca(NO_3)_2$ and $MgSO_4$, the following salts were used: KH_2PO_4, $(NH_4)_2SO_4$, $Ca(NO_3)_2$ and $MgSO_4$, or the place of KNO_3 was taken by $(NH_4)_2SO_4$, thus partially eliminating the potassium and nitrogen, and introducing an entirely new ion, NH_4. The purpose of this substitution, as was stated in the introduction, was to find out whether or not a new ionic group, which adds no new element to the culture solution, might influence the crop yield in the sand cultures. Iron was supplied in the form of a small amount of iron rust.

The total osmotic concentration of the nutrient solutions used was about 2.5 atmospheres in terms of possible osmotic pressure. The calculations for volume-molecular concentration of ammonium sulfate were made according to the method adopted by Tottingham, using the dissociation figures from Jones' tables (12). The stock solutions were prepared in M/4 concentration. The needed amount of solution of the different salts was drawn into a small volume of distilled water in a 250-cc. volumetric flask which was then filled to the mark. The number of cubic centimeters of different stock solutions necessary for obtaining the nutrient solution with the osmotic concentration of approximately 2.5 atmospheres is given for convenience in table 1.

The plants were started in moist sand, and, when about 2 cm. in height, were selected for uniformity and transplanted into the culture pots. Three healthy seedlings were used in each pot. After transplanting, the surface of the sand, as mentioned above, was sealed with paraffine.

Forty sand cultures, twenty in each of the two series thus prepared, were set on January 25, 1917, and continued to March 5, the total growing period in pots being 39 days. Later the experiment was repeated, and was run from April 2 to May 11 of the same year, again making 39 days for the growing season. The plants, when harvested, were in full bloom, while on the most vigorous ones the pods were being formed.

RESULTS AND DISCUSSION

Concentration of the nutrient solution

In order to ascertain whether an appreciable adsorption of salts had taken place after adding the solutions of these salts to the sand, the concentration of the solutions was determined both before and after adding them to the sand. Moreover, the concentration of the solutions in plant cultures also was deter-

mined. This was done at three different times during the growth: (a) at the beginning of the experiment, (b) 15 days after the start, and (c) at the end of the 39-day period of growth. A portion of the solution was withdrawn by means of suction from each of the sand cultures. This was done at the time of renewing the solutions. In addition to this, the concentration of the moist sand at the end of the experiment also was determined by means of the cryoscopic method. The concentration of the solution in the sand was determined by the method suggested by Bouyoucos and McCool (2), and the calculations were made with the aid of data gathered by Harris and Gortner (10).

Table 2 gives the data obtained by these determinations with the Tottingham series while table 3 presents the similar data for the ammonium sulfate series. The arrangement of the data is the same in both tables. The first two columns represent the number of cultures in the triangles and the laboratory numbers, respectively. The following seven columns give the degrees (Centigrade) of depression of the freezing point, while the last seven columns show the corresponding concentrations in atmospheres. Studying these tables carefully one notices two very significant features. One of these lies in the fact that, with few exceptions, the values for the original solution in both series, for the sand treated with solution, for all solutions obtained by suction from the culture pots, and, finally, for the sand at the end of the experiment, follow one another with striking consistency. There is no great difference between the concentration of the original solution and that of the solution in the sand cultures. In the Tottingham series the values for solutions obtained from the sand cultures are in very close agreement with the values of the original solution and sand treated with it. The figures for the sand at the end of the experiment, however, are lower than those of others, but this latter feature is not so noticeable in the series containing ammonium sulfate. Thus, it is evident that Livingston's (15) results, as emphatically quoted by Breazeale (3), could not be explained on the basis of total concentration. His results seem to indicate that while the best concentration of solution in water cultures for plant growth is around 300 parts per million, the corresponding best concentration in sand cultures lies in the neighborhood of 2500 parts per million. Unless very fine sand is used and the most of the salts composing the solution are similar in their behavior to phosphates, no such reduction in the effective concentration is to be expected. In this connection the work of Bouyoucos and McCool (2) with a number of soils and sand which they treated with different salts of $\frac{N}{10}$ concentration, is of special interest. Testing the total concentration directly in the soil treated with the salt solutions, Bouyoucos and McCool obtained results which show that different salts are adsorbed to a different degree by the same soil, and that the different soils and sands have different powers of adsorption. The adsorption of sand, which they treated with $\frac{N}{10}$ salt solution, was very small. However, when the solution of a salt is sufficiently diluted, the relative adsorption of even coarse sand becomes considerable and that of the fine sand adsorption is very great, as was shown by

TABLE 2

Freezing-point depression and the concentration in atmospheres of Tottingham's solutions

POSITION	NUMBER	FREEZING-POINT DEPRESSION: Original solutions	FREEZING-POINT DEPRESSION: Sand treated with solutions	FREEZING-POINT DEPRESSION: Difference	FREEZING-POINT DEPRESSION: Solution obtained by suction from pots at the beginning of experiment	FREEZING-POINT DEPRESSION: Solution obtained by suction from pots 15 days after beginning	FREEZING-POINT DEPRESSION: Solution obtained by suction at the end of the experiment	FREEZING-POINT DEPRESSION: Sand in pots at the end of the experiment	CONCENTRATION IN ATMOSPHERES: Original solution	CONCENTRATION IN ATMOSPHERES: Sand treated with original solution	CONCENTRATION IN ATMOSPHERES: Difference	CONCENTRATION IN ATMOSPHERES: Solution obtained by suction from pots at the beginning of the experiment	CONCENTRATION IN ATMOSPHERES: Solution obtained by suction from pots 15 days after beginning of experiment	CONCENTRATION IN ATMOSPHERES: Solution obtained by suction from pots at the end of the experiment	CONCENTRATION IN ATMOSPHERES: Sand in pots at the end of the experiment
		°C.	°C.	°C.	°C.	°C.	°C.	°C	atm.	atm.	atm.	atm.	atm.	atm.	atm.
$T_1R_1C_1$	1	0.169	0.154	0.015	0.161	0.185	0.159	0.111	2.04	1.86	0.18	1.94	2.23	1.92	1.31
R_1C_3	2	0.180	0.178	0.002	0.173	0.197	0.166	0.150	2.17	2.15	0.02	2.09	2.38	2.00	1.81
R_1C_5	3	0.184	0.169	0.015	0.180	0.173	0.173	0.148	2.22	2.04	0.18	2.17	2.09	2.09	1.79
R_1C_7	4	0.208	0.205	0.003	0.200	0.201	0.191	0.178	2.51	2.47	0.04	2.41	2.42	2.30	2.15
R_3C_1	5	0.179	0.155	0.024	0.168	0.185	0.165	0.146	2.16	1.87	0.29	2.03	2.23	1.99	1.76
R_3C_3	6	0.172	0.145	0.027	0.162	0.173	0.167	0.155	2.07	1.75	0.32	1.95	2.09	2.01	1.87
R_3C_5	7	0.201	0.201	0.000	0.194	0.203	0.188	0.175	2.42	2.42	0.00	2.34	2.44	2.27	2.11
R_5C_1	8	0.183	0.171	0.012	0.163	0.163	0.158	0.144	2.21	2.06	0.15	1.97	1.97	1.91	1.74
R_5C_3	9	0.208	0.198	0.010	0.174	0.198	0.181	0.155	2.51	2.39	0.12	2.10	2.39	2.18	1.87
R_7C_1	10	0.201	0.197	0.004	0.171	0.193	0.164	0.151	2.42	2.38	0.04	2.06	2.33	1.98	1.82
$T_3R_1C_1$	11	0.176	0.172	0.004	0.182	0.190	0.166	0.146	2.12	2.07	0.05	2.20	2.29	2.00	1.76
R_1C_3	12	0.173	0.165	0.008	0.179	0.195	0.170	0.150	2.09	1.99	0.10	2.16	2.35	2.05	1.81
R_1C_5	13	0.206	0.199	0.007	0.188	0.199	0.180	0.180	2.48	2.40	0.08	2.27	2.40	2.17	2.17
R_3C_1	14	0.171	0.162	0.009	0.185	0.178	0.160	0.130	2.06	1.95	0.11	2.22	2.15	1.93	1.46
R_3C_3	15	0.195	0.193	0.002	0.198	0.196	0.184	0.160	2.35	2.33	0.02	2.39	2.36	2.22	1.93
R_5C_1	16	0.192	0.191	0.001	0.196	0.185	0.181	0.216	2.32	2.30	0.02	2.36	2.23	2.18	2.60
$T_5R_1C_1$	17	0.175	0.169	0.006	0.166	0.164	0.157	0.161	2.11	2.04	0.07	2.00	1.98	1.89	1.94
R_1C_3	18	0.199	0.187	0.012	0.193	0.200	0.182	0.174	2.40	2.26	0.14	2.33	2.41	2.20	2.10
R_3C_1	19	0.189	0.190	−0.001	0.199	0.194	0.188	0.184	2.28	2.29	−0.01	2.40	2.34	2.27	2.22
$T_7R_1C_1$	20	0.200	0.199	0.001	0.194	0.200	0.181	0.175	2.41	2.40	0.01	2.34	2.41	2.18	2.11
Average		0.188	0.180	0.008	0.181	0.189	0.173	0.161	2.27	2.17	0.10	2.18	2.28	2.09	1.94

TABLE 3

Freezing-point depression and concentration in atmospheres of solutions of the ammonium sulfate series

POSITION	NUMBER	FREEZING-POINT DEPRESSION							CONCENTRATION IN ATMOSPHERES						
		Original solutions	Sand treated with solutions	Difference	Solution obtained by suction from pots at the beginning of experiment	Solution obtained by suction from pots 15 days after beginning	Solution obtained by suction from pots at the end of experiment	Sand in pots at the end of the experiment	Original solution	Sand treated with original solution	Difference	Solution obtained by suction from pots at the beginning of experiment	Solution obtained by suction from pots 15 days after the beginning of experiment	Solution obtained by suction from pots at the end of experiment	Sand in pots at the end of experiment
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	atm.	atm.	atm.	atm.	atm.	atm.	atm.
$T_1R_1C_1$	21	0.169	0.183	−0.014	0.174	0.196	0.168	0.201	2.04	2.21	−0.17	2.10	2.36	2.03	2.42
C_3	22	0.179	0.186	−0.007	0.190	0.205	0.186	0.223	2.16	2.24	−0.08	2.47	2.47	2.24	2.69
C_5	23	0.179	0.201	−0.022	0.188	0.175	0.182	0.172	2.16	2.42	−0.26	2.27	2.10	2.20	2.07
C_7	24	0.204	0.224	−0.020	0.198	0.225	0.203	0.164	2.46	2.70	−0.24	2.39	3.10	2.45	1.98
R_3C_1	25	0.167	0.205	−0.038	0.174	0.185	0.158	0.195	2.01	2.47	−0.46	2.10	2.23	1.91	2.35
C_3	26	0.174	0.202	−0.028	0.176	0.190	0.170	0.166	2.10	2.44	−0.31	2.12	2.29	2.05	2.00
C_5	27	0.196	0.204	−0.008	0.202	0.215	0.195	0.199	2.36	2.46	−0.10	2.44	2.59	2.35	2.40
R_5C_1	28	0.168	0.168	0.000	0.165	0.178	0.163	0.162	2.03	2.03	0.00	1.99	2.15	1.97	1.95
C_3	29	0.188	0.205	−0.017	0.198	0.197	0.191	0.215	2.27	2.47	−0.20	2.39	2.38	2.30	2.59
R_7C_1	30	0.167	0.184	−0.017	0.189	0.200	0.183	0.184	2.01	2.22	−0.21	2.28	2.41	2.21	2.22
$T_3R_1C_1$	31	0.174	0.193	−0.019	0.185	0.197	0.174	0.129	2.10	2.33	−0.23	2.23	2.38	2.10	1.45
C_3	32	0.174	0.179	−0.005	0.182	0.198	0.180	0.162	2.10	2.16	−0.06	2.20	2.39	2.17	1.95
C_5	33	0.198	0.205	−0.007	0.199	0.230	0.198	0.211	2.39	2.47	−0.08	2.40	2.77	2.39	2.54
R_3C_1	34	0.173	0.183	−0.010	0.176	0.192	0.166	0.191	2.09	2.21	−0.12	2.12	2.32	2.00	2.30
C_3	35	0.201	0.221	−0.020	0.178	0.208	0.194	0.224	2.42	2.66	−0.16	2.15	2.51	2.34	2.70
R_5C_1	36	0.200	0.191	0.009	0.182	0.208	0.182	0.231	2.41	2.30	0.11	2.20	2.51	2.20	2.21
$T_5R_1C_1$	37	0.158	0.148	0.010	0.155	0.211	0.171	0.183	1.91	1.79	0.12	1.87	2.54	2.06	2.21
C_3	38	0.194	0.176	0.018	0.187		0.192	0.201	2.34	2.12	0.22	2.25		2.32	2.42
R_3C_1	39	0.181	0.184	−0.003	0.190	0.197	0.180	0.214	2.18	2.22	−0.04	2.29	2.38	2.17	2.58
$T_7R_1C_1$	40	0.193	0.184	0.009	0.186	0.214	0.192	0.200	2.33	2.22	0.11	2.24	2.58	2.32	2.41
Average..............		0.177	0.191	−0.014	0.184	0.200	0.176	0.176	2.13	2.30	−0.17	2.22	2.41	2.12	2.12

Wolkoff (33) in the preliminary paper on the study of adsorption of ammonium sulfate at different concentrations.

Another outstanding feature of the results submitted in tables 2 and 3 and graphically presented in figure 3, is the apparent tendency of the nutrient solution containing ammonium sulfate to become more concentrated than the original solution, when added to the sand. In fifteen cases out of twenty the freezing-point depression of the solution in sand was greater than that of the corresponding original solution. The behavior of the Tottingham solution was entirely different in this respect. In nineteen cases out of twenty the concentration of the solution in sand was somewhat smaller than the concentration of the solution before adding this sand. The solutions used in this particular experiment were prepared together and used at once. The same stock solutions were employed for the determination of concentration of the

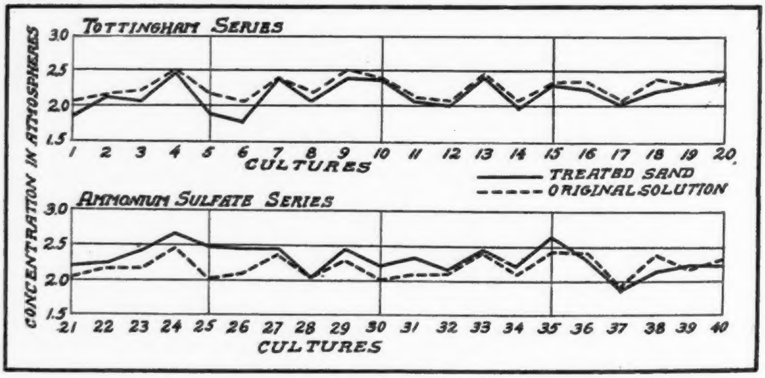

FIG. 3. THE CONCENTRATION OF TOTTINGHAM NUTRIENT SOLUTION AND SOLUTION OF AMMONIUM SULFATE SERIES BEFORE AND AFTER ADDING THESE SOLUTIONS TO A WASHED SEA SAND

solution as such and when added to the sand. Duplicates, of course, were made in every instance. Besides, in the cases where the differences between the original solutions and the solutions in sand were very pronounced, the experiment was repeated. No evident error was revealed. These results are in good agreement with the data obtained by the author (33) working with ammonium sulfate solutions and sand of different fineness. Evidently, when ammonium sulfate is present in combination with other salts, it has a similar effect on the total concentration of the solution as it has when used singly, although the degree of the effect produced is modified by the presence of other salts and also by the salt proportions. The explanation for this interesting phenomenon is offered by the author in another paper (33). It seems that when sand or soil is treated with ammonium sulfate, the alkali radical, NH_4, is adsorbed by this solid phase more strongly than the acid radical, SO_4. As a

result of this unequal adsorption some excess of the SO_4-ion is left in solution, and, uniting with H_2-ions of slightly dissociated water, forms some free H_2SO_4. Since the dissociation of acid is greater than that of the original salt, ammonium sulfate, the freezing-point depression in the case of sand will be greater, although the total (absolute) concentration of the solution may be slightly reduced by the adsorption. This phenomenon is more pronounced in the sand with a lower per cent of moisture than with a higher one. In other words, where the solid phase is relatively reduced and the relative surface is correspondingly diminished, the effect of adsorption becomes smaller. This fact, of course, is in accord with the theory of adsorption. In the case of soil (33) treated with ammonium sulfate, however, no such apparent increase in total concentration was noticed by this method, because the effect of adsorption is greater than that of any increase in dissociation due to the formation of acid.

This phenomenon of increase in concentration of the salt solution on adding it to the sand is similar to that which was first observed by Graham (8) in 1830, but attributed by him to experimental error. The fact of increase of concentration of some salt solutions after adding them to some solid phase, such as charcoal, kaolin, clay, or powdered sand, is now well established by Gore (7), Lagergren (14), William (32) and quite recently by McCall and his co-workers (16). Dealing with the solid phase greatly divided to offer an enormous surface energy, the above-mentioned authors evidently had the adsorption of water out of the liquid phase to such an extent that it caused an increase in concentration of the remaining salt solution. The phenomenon is called either "negative adsorption" (Lagergren, William), or "special case of selective adsorption" (McCall). In the experiments described in the present work, the phenomena observed were evidently due to a selective adsorption of one of the ions and subsequent formation of acid out of the acid radical, and the hydrogen ion of the slightly ionized water. It is a pure case of a physico-chemical process that may take place in the heterogenous system.

RESULTS OF THE MAIN EXPERIMENT

Introductory remarks

The effect of the introduction of ammonium sulfate into Shive's nutrient solution, or of the substitution of this salt for potassium nitrate in Tottingham's nutrient solution, on the plant growth of soybeans was studied by means of several criteria; (a) the general appearance of plants, (b) dry weight of tops, (c) dry weight of roots, (d) transpiration, (e) water requirement of tops, and (f) water requirement of roots.

General appearance of plants of the two series

The plants of the ammonium sulfate series appeared, in general, more healthy, more bushy and slightly taller, than the plants in the corresponding cultures of the Tottingham series. The most pronounced difference was in

the color of the plants of the two series, the plants of the ammonium sulfate series being much greener than those of the Tottingham series.

At the beginning of the growing period, plants with ammonium sulfate as a part of their nutrient solution appeared to be injured and were retarded in their growth for a week or ten days. This feature, however, gradually disappeared, and the plants began to make a vigorous growth.

Just before harvesting the plants of the first experiment, on March 5, observations on the cultures were made, noting the injury, if any, and the degree

TABLE 4

Injury of plants in Tottingham and ammonium sulfate series of the first experiment (the observations were made just before harvesting the plants.)

TOTTINGHAM SERIES	AMMONIUM SULFATE SERIES
1. Two plants slightly injured	21. No injury
2. No injury	22. No injury
3. No injury	23. No injury
4. No injury	24. No injury
5. Severe injury of all plants	25. Very slight injury
6. Two plants slightly injured	26. No injury
7. Two plants slightly injured	27. No injury
8. Severe injury of all plants	28. Considerable injury, all plants stunted
9. Apparent injury slight, but plants stunted	29. No injury
10. Severe injury, plants stunted	30. Considerable injury; all plants stunted
11. No injury	31. No injury
12. No injury	32. No injury
13. Plants conspicuously yellow but no apparent injury noticed	33. No injury
14. Severe injury	34. One plant died from mechanical injury; otherwise, no injury
15. Slight injury	35. No injury
16. Severe injury	36. Very slight injury
17. Slight injury	37. No injury
18. Slight injury	38. Four leaves of one plant wilted around the margins
19. Severe injury	39. No injury
20. Severe injury; leaf edges wilted, as if burned	40. No injury

of the injury. Each culture was judged as a whole. Brief memoranda are given in table 4.

A glance at the memoranda given in table 4 reveals the fact that, with the exception of cultures 28 and 40, the plants of the ammonium sulfate series suffered much less injury than the corresponding ones of the Tottingham series.

In the second experiment, which was conducted between April 2 and May 11, these differences were more pronounced; that is to say, the difference between the best culture in the ammonium sulfate series and the best culture of the Tottingham series in the second experiment was much greater than in the first experiment. On the other hand, the difference between the poorest cul-

tures of the two series in both experiments was also more pronounced during the second experiment. The injury of the plants of cultures 28 and 30 in the second experiment was such that nearly all plants died in the course of two weeks; while the luxuriant growth of culture 32 was more conspicuous in comparison with the growth of the plants in the corresponding Tottingham's culture, No. 12. The difference in the behavior of the plants grown at different times of the year is attributed, at least partly, to the difference in the temperature of the greenhouse. This temperature, on the average, was much higher during the second experiment than during the first one. Since all the chemical reactions proceed with a greater speed at an elevated temperature than at a lower one, the difference in the effect of the salts in different proportions at different temperatures could easily be explained on these grounds.

Outside of this feature, the general trend of the growth of plants in both experiments was practically the same, as one will notice in studying the data of the dry weight of tops and roots in both series which will be shortly presented.

Dry weight of tops

The dry weight of tops was obtained by cutting the plants at the surface of the sand, and drying them in weighed, wide-mouthed bottles in the electric oven at about 108°C. until constant weight was attained. The results are given in table 5. They show the dry weight of tops secured in the first and the second experiments, and the average for the two. The relative values also are given. The first four columns of the table present the data of the Tottingham series, while the last four present the corresponding values for the ammonium sulfate series. The relative values were obtained by dividing each number by the average dry weight of the two series of both experiments, this average figure being 2.3881 gm. These relative values are plotted in figure 4, following the general scheme (with minor modifications) of representation as suggested by Tottingham (29) and also by Shive (26). This chart shows the cultures in their proper places on the triangles. The cultures whose relative values are 110 or above, are shaded by small crosses, while the portion of the triangles with cultures of values of 90 or lower are shaded by small circles. The examination of the average data in table 5 and figure 4 show striking differences between the corresponding values of the cultures in the two series. The best yields in the Tottingham series were obtained from cultures $T_3R_1C_1$ and $T_3R_1C_3$ with the salt proportions corresponding to 0.0156 m. KH_2PO_4, 0.0049 m. KNO_3, 0.0036 m. Ca $(NO_3)_2$, and 0.00290 m. $MgSO_4$ in the first case, and 0.0156 m. KH_2PO_4, 0.0049 m. KNO_3, 0.0108 m. $Ca(NO_3)_2$ and 0.0174 m. $MgSO_4$ in the second case. The best culture of the ammonium sulfate series was $T_3R_1C_1$, or that which contained 0.0156 m. KH_2PO_4, 0.0036 m. $(NH_4)_2SO_4$, 0.0108 m. $Ca(NO_3)_2$, and 0.0174 m. $MgSO_4$. The poorest culture in both series was $T_1R_7C_1$. It contained the greatest amount of KNO_3 in the Tottingham series and the corresponding amount of $(NH_4)_2SO_4$ in the

ammonium sulfate series. The best culture of the ammonium sulfate series, however, was considerably better than the best culture of the Tottingham series, while of the poorest cultures of the two series the culture of Tottingham's solution was better. The variation between the best and the poorest cultures was much greater in the ammonium sulfate series than in the Tottingham series.

The plant growth of the cultures containing ammonium sulfate, was much

TABLE 5

Dry weight of tops of soybeans grown in sand cultures of the Tottingham and ammonium sulfate series

POSITION	TOTTINGHAM SERIES					AMMONIUM SULFATE SERIES				
	Number	Trial 1	Trial 2	Average	Relative value	Number	Trial 1	Trial 2	Average	Relative value
		grams	grams	grams			grams	grams	grams	
$T_1R_1C_1$	1	2.3390	2.6975	2.5183	106	21	2.2330	3.8747	3.0539	126
R_1C_3	2	2.4716	2.5170	2.4942	101	22	2.6100	4.9817	3.7959	156
R_1C_5	3	2.3340	2.1323	2.2332	95	23	2.5575	4.7962	3.6769	151
R_1C_7	4	2.3322	1.6865	2.0094	86	24	2.6150	4.7812	3.6981	152
R_3C_1	5	1.7714	2.4997	2.1356	89	25	1.4833	2.2685	1.8759	78
R_3C_3	6	2.4210	2.0950	2.2580	96	26	2.0996	4.2710	3.1853	131
R_3C_5	7	2.0846	1.6547	1.8697	80	27	2.1760	3.6059	2.8910	119
R_5C_1	8	1.8292	2.1405	1.9849	83	28	1.4807	0.3798	0.9303	42
R_5C_3	9	1.9480	1.3702	1.6591	72	29	1.8512	2.7548	2.3030	96
R_7C_1	10	1.3316	1.6507	1.6412	63	30	1.2014	0.5235	0.8624	37
$T_3R_1C_1$	11	2.5590	2.7285	2.6438	112	31	2.7491	3.5485	3.1488	132
R_1C_3	12	2.7210	2.4598	2.5904	112	32	3.1743	5.2335	4.2039	173
R_1C_5	13	1.8848	1.5540	1.7174	74	33	2.5979	3.4932	3.0456	127
R_3C_1	14	2.1020	2.1152	2.1086	90	34	2.0410	2.6805	2.3608	99
R_3C_3	15	1.9934	2.0113	2.0044	85	35	2.2241	3.7773	3.0007	124
R_5C_1	16	1.7570	2.0312	1.8941	80	36	1.3306	1.3877	1.3592	58
$T_5R_1C_1$	17	2.4636	2.1007	2.2822	97	37	2.6422	3.2525	2.9474	124
R_1C_3	18	2.2678	1.7015	1.9847	86	38	2.5464	2.8220	2.6843	114
R_3C_1	19	1.8850	2.2182	2.0516	87	39	1.9795	2.0187	1.9991	85
$T_7R_1C_1$	20	2.2200	2.5140	2.3670	100	40	2.1805	2.6370	2.4088	101
Average					89.5	Average				111

better, on the whole, than that of the cultures without this salt. This fact is very well illustrated by figure 4. Indeed, only two cultures in the Tottingham series gave values above 110, while the large shaded portion with crosses on the ammonium sulfate side shows that twelve out of a total of twenty cultures exceeded this value. On the other hand, while twelve cultures of the Tottingham series fell on or below 90, only five cultures of the ammonium sulfate series were found below this value. Consequently, very few cultures were located between the high and the low areas in the case of the ammonium

sulfate series, as compared with the Tottingham series. There is a pronounced tendency for the cultures of the ammonium sulfate series to show considerable

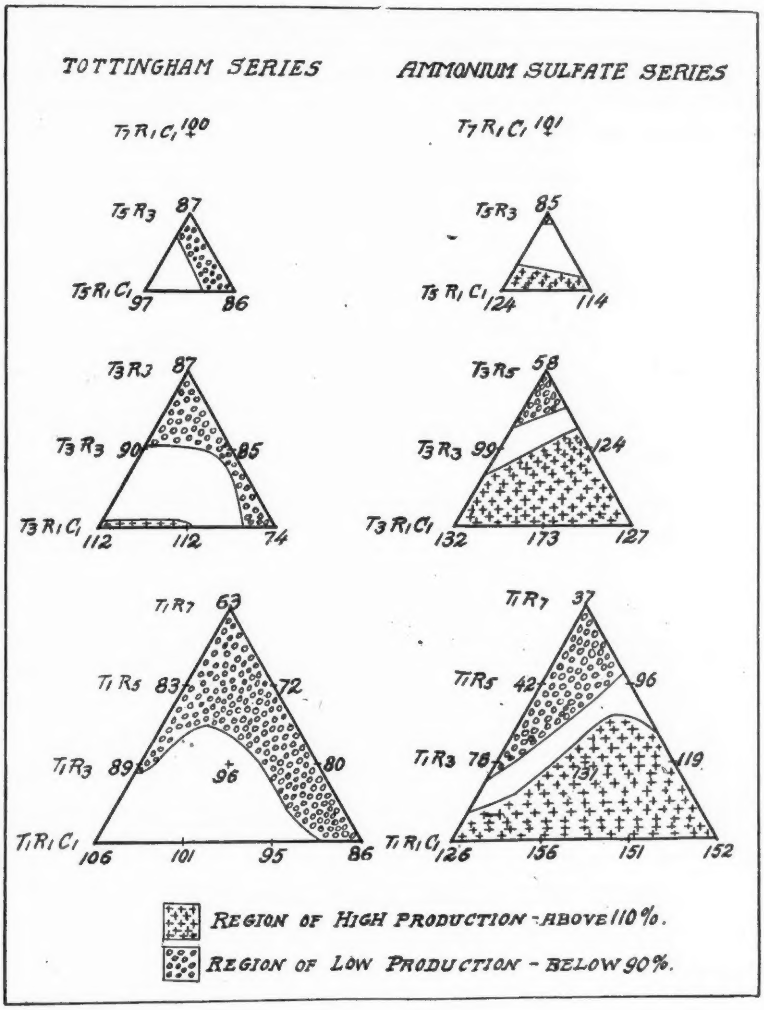

FIG. 4. THE RELATIVE AVERAGE VALUES FOR TOP YIELDS OF SOYBEANS OF TOTTINGHAM AND AMMONIUM SULFATE SERIES

The high and low yield regions are shaded with crosses and circles, respectively.

fluctuation upon a slight alteration of the salt proportions. The fluctuations in this respect are not so striking in the Tottingham series.

Dry weight of roots

The dry weight of roots was obtained by washing out the sand from the roots by means of a small stream of water on a fine screen and drying the roots in the electric oven. Since it is an impossible task to wash the sand from the roots, the weight of organic matter of the roots was obtained in an indirect way. The washed roots were burned in a platinum dish. The difference between the weight of the dish with the material before and after the burning was taken as the weight of the roots. No corrections were made for the ash content.

TABLE 6

Dry weight of roots of soybeans grown in sand cultures of the Tottingham and ammonium sulfate series

POSITION	TOTTINGHAM SERIES					AMMONIUM SULFATE SERIES				
	Number	Trial 1	Trial 2	Average	Relative value	Number	Trial 1	Trial 2	Average	Relative value
		gram	*gram*				*gram*	*gram*		
$T_1R_1C_1$	1	0.4000	0.4210	0.4105	108	21	0.4590	0.4334	0.4462	117
R_1C_3	2	0.4092	0.4403	0.4248	112	22	0.5868			
R_1C_5	3	0.4536	0.4335	0.4436	116	23	0.4902	0.7275	0.6089	160
R_1C_7	4	0.4436	0.3800	0.4118	108	24	0.3630	0.6405	0.5018	182
R_3C_1	5	0.3132	0.4057	0.3595	95	25	0.2874	0.2305	0.2590	68
R_3C_3	6	0.4628	0.2068	0.3348	88	26	0.4392	0.5745	0.5069	133
R_3C_5	7	0.3472	0.2553	0.3013	79	27	0.3314	0.4101	0.3708	98
R_5C_1	8	0.3118	0.3981	0.3550	94	28	0.2190	0.0398	0.1294	34
R_5C_3	9	0.3230	0.2667	0.2999	78	29	0.2824	0.2322	0.2573	63
R_7C_1	10	0.2296	0.3952	0.3124	82	30	0.1436	0.0410	0.0923	25
$T_3R_1C_1$	11	0.3950	0.4510	0.4230	112	31	0.5118	0.4283	0.4701	124
R_1C_3	12	0.4728	0.4443	0.4586	121	32	0.5616	0.7948	0.6782	179
R_1C_5	13	0.3620	0.3795	0.3708	98	33	0.4864	0.4680	0.4772	125
R_3C_1	14	0.3700	0.3857	0.3779	99	34	0.2500	0.3365	0.2933	78
R_3C_3	15	0.3638	0.3428	0.3533	93	35	0.4350	0.4525	0.4438	117
R_5C_1	16	0.2838	0.3805	0.3322	88	36	0.2132	0.1393	0.1763	47
$T_5R_1C_1$	17	0.4850	0.3575	0.4213	111	37	0.4676	0.4252	0.4464	118
R_1C_3	18	0.4130	0.2960	0.3545	93	38	0.4422	0.4133	0.4278	113
R_3C_1	19	0.3324	0.4483	0.3904	103	39	0.2894	0.2108	0.2501	66
$T_7R_1C_1$	20	0.3718	0.4105	0.3912	103	40	0.4926	0.2873	0.3900	103
Average					99	Average				101

The dry weight of roots, as presented in table 6 and figure 5, shows, in general, the same main differences between the two series, as were pointed out in the case of the tops.

The culture producing the best root yield in the Tottingham series was $T_3R_1C_3$, or one of the two best cultures for tops of the same series. It was

the best culture for roots and tops in the ammonium sulfate series. The poorest culture for roots in the ammonium sulfate series was $T_1R_7C_1$, or the same

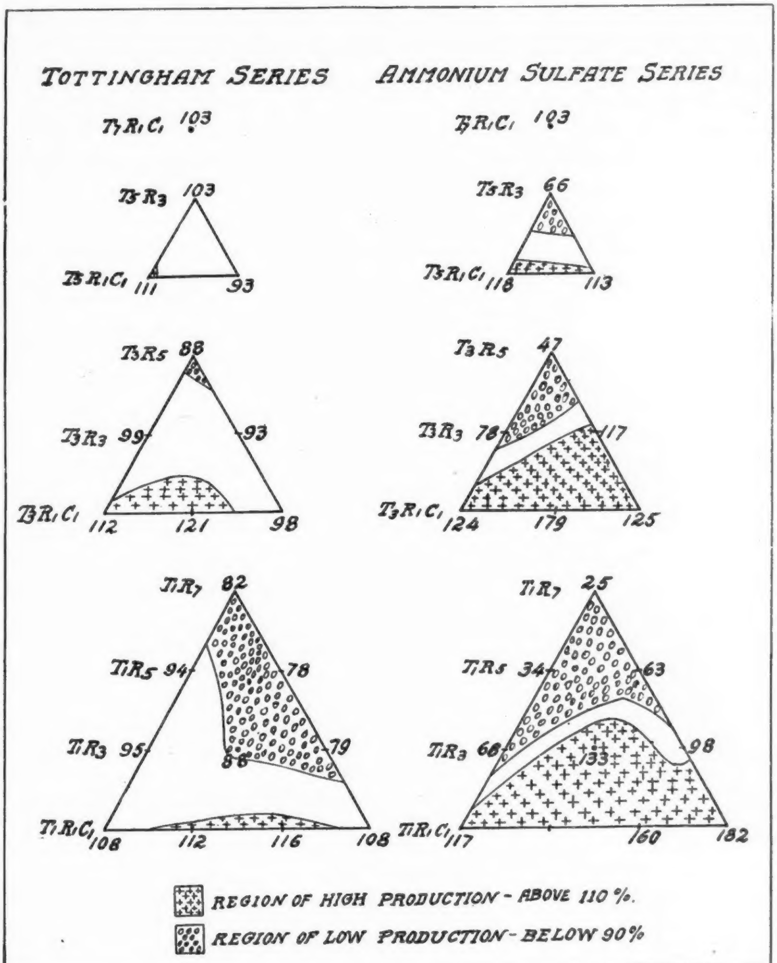

FIG. 5. THE RELATIVE AVERAGE VALUES FOR ROOT YIELDS OF SOYBEANS OF TOTTINGHAM AND AMMONIUM SULFATE SERIES

The high and low yield regions are shaded with crosses and circles, respectively.

as the poorest for the tops in the same series. The poorest culture in the Tottingham series was $T_1R_5C_3$, which was next to the poorest in the case of tops.

The region of high yields on the triangles for roots corresponds fairly well with the region of high yields on the triangles for tops; also, the areas of poor yields of roots approximately coincide with the areas of low yields of tops.

The ammonium sulfate series contains eleven cultures whose relative values are above 110, while only five cultures of the Tottingham series lie above that value. The relation is nearly reversed, however, when the number of cultures producing poor root yields is considered, or those whose relative values are below 90. In the ammonium sulfate series there are seven, but in the Tottingham series there are only five. It seems that the ammonium sulfate in the nutrient solution in the sand cultures caused a relatively better growth of

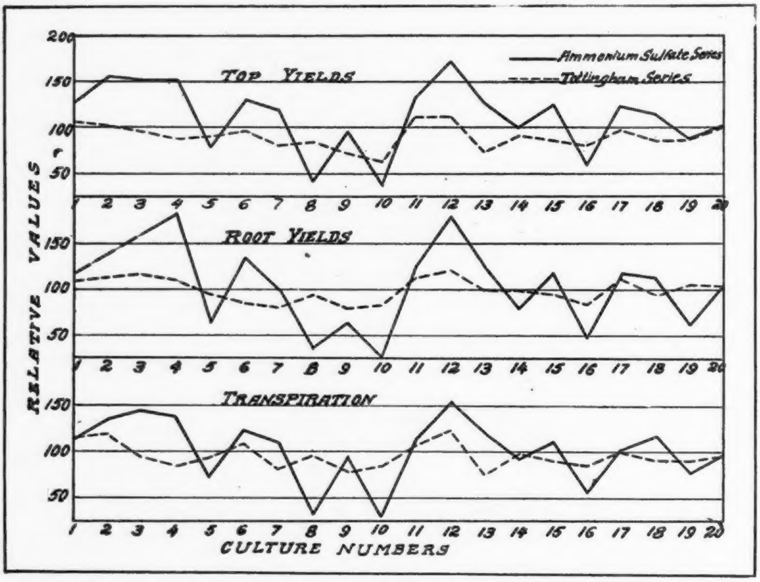

FIG. 6. RELATIVE VALUES FOR TOP YIELDS, ROOT YIELDS AND TRANSPIRATION OF WATER BY PLANTS OF SOYBEANS IN TOTTINGHAM AND AMMONIUM SULFATE SERIES

tops than of roots, while a reverse influence is noticed in the Tottingham series. This relation is plainly seen from the average figures of the relative values in tables 5 and 6, which are, in the ammonium sulfate series, 111 for tops and only 101 for roots. In the Tottingham series these figures are 89 and 99, respectively. In other words, the average root development in the different salt proportions of the ammonium sulfate series was quite large as compared with that of the Tottingham series. In this respect the data for roots and for tops show a very close agreement. Indeed, the relation between the growth of tops and that of roots in the same series is noticed throughout, as one can judge by studying the relative values in tables 5 and 6, and also in figure 6. With the

variation in the salt proportions from one culture to another, the curve for root yields of the ammonium sulfate series follows very closely the curve for the top yields of the same series. The relation between the root and top yields is also very similar in the Tottingham series, though these features are less pronounced than in the ammonium sulfate series. The correlation between the development of tops and that of roots is especially interesting, because it is not always found to exist with different plants. For instance, Tottingham (29), working with wheat in water cultures, could find no relation whatsoever between the dry weight of tops and that of roots. Shive (26) could trace some relations between yields of tops and roots. McCall (19) repeated Shive's work, carrying it along side of sand cultures, and published results that show very little correlation between the dry weight of tops and that of roots in both optimum series of Shive's water and sand cultures. Later, working with buckwheat in water cultures, Shive (27) found a fair relation between the growth of these two parts of the plant. Evidently, different plants behave differently as influenced by different salt proportions in the nutrient medium.

Transpiration and water requirement

The transpiration data were obtained by keeping a record of the water evaporated through the plants. In order to bring out clearly the relations between transpiration and the growth of tops and that of roots, the data of table 7 are presented. In both series the curves for transpiration data closely follow the curves of top yields and also of root yields. The relation of the transpiration of the roots to that of the tops, however, is more close in the case of the ammonium sulfate series than in that of the Tottingham series. In the case of the relation of transpiration to growth of tops, the results are well in accord with the results of some of the previous investigators, among whom could be mentioned Whitney and Cameron (31), Livingston, Britton and Reid (15), and especially Livingston (16 and 17), Shive (26) and McCall (19). Theoretically, the amount of water transpired, the moisture content of the soil and the meteorological conditions remaining the same, is mainly dependent upon both the leaf surface of the plant and the surface of the active root hairs. The influence of the leaf area, of course, is predominant. Some secondary factors, may play a prominent part only under some abnormal conditions of the plant growth.

The water requirement of the tops was calculated by dividing the total water loss in grams by the dry weight of tops. The data, together with their relative values, obtained by taking the average water requirement of both series as 100, are given in the table 7. The corresponding data for roots are presented in the same table. The results show the water requirement per gram of dry weight of tops in the Tottingham series to lie between 327 gm. in culture $T_3R_1C_1$ (the best culture for the top growth) and 387 gm. in culture

TABLE 7

Average transpiration, water requirement of tops and water requirement of roots, together with their relative values, of soybeans grown in Tottingham nutrient solution and the nutrient solution with ammonium sulfate in sand cultures

POSITION	NUMBER	TOTTINGHAM SERIES								NUMBER	AMMONIUM SULFATE SERIES							
		Transpiration				Tops		Roots			Transpiration				Tops		Roots	
		Trial 1	Trial 2	Average	Relative value	Grams of water required per gram of dry matter	Relative value	Grams of water required per gram of dry matter	Relative value		Trial 1	Trial 2	Average	Relative value	Grams of water required per gram of dry matter	Relative value	Grams of water required per gram of dry matter	Relative value
		grams	*grams*	*grams*							*grams*	*grams*	*grams*					
$T_1R_1C_1$	1	699	1156	926	114	369	108	2560	123	21	711	1110	911	112	298	87	2040	98
R_1C_3	2	780	1123	952	117	382	112	2240	108	22	737	1449	1093	134	287	84		
R_1C_5	3	735	798	767	94	344	101	1730	83	23	768	1477	1123	138	305	89	1845	89
R_1C_7	4	735	688	712	87	355	104	1730	83	24	748	1448	1093	134	292	85	2188	105
R_3C_1	5	570	964	767	94	360	105	2135	103	25	500	652	576	71	307	90	2223	107
R_3C_3	6	841	897	869	107	385	113	2595	125	26	709	1264	987	121	310	91	1945	94
R_3C_5	7	639	652	646	79	345	101	2143	103	27	665	1123	894	110	310	91	2420	117
R_5C_1	8	619	916	768	94	387	113	2160	104	28	438	92	265	32	285	83	2055	99
R_5C_3	9	687	573	630	77	380	111	2100	101	29	607	909	758	93	330	97	2950	142
R_7C_1	10	464	693	679	83	353	103	1853	89	30	366	136	251	31	192	56	2720	131
$T_3R_1C_1$	11	697	1028	863	106	327	96	1998	96	31	788	1062	925	114	294	86	1970	95
R_1C_3	12	823	1168	996	122	385	113	2170	104	32	921	1581	1251	154	298	87	1845	89
R_1C_5	13	611	563	587	72	342	100	1580	76	33	694	1233	964	118	316	92	2020	97
R_3C_1	14	673	869	771	95	366	107	2280	110	34	586	879	733	90	311	91	2515	121
R_3C_3	15	637	802	720	88	360	105	2085	100	35	723	1274	899	110	300	88	2025	98
R_5C_1	16	542	830	686	84	362	106	2065	99	36	416	475	446	55	328	96	2525	122
$T_5R_1C_1$	17	773	823	798	98	350	102	1895	91	37	771	870	821	101	378	81	1840	89
R_1C_3	18	758	712	735	90	370	108	2073	100	38	800	1095	948	116	354	104	2215	107
R_3C_1	19	592	866	729	90	356	101	1868	90	39	571	653	612	75	306	90	2450	118
$T_7R_1C_1$	20	678	853	766	94	324	95	1955	94	40	686	854	770	95	320	94	1975	95
Average....................................						361	105.5	2060	99.3						322	94.5	2088	100.7

$T_1R_5C_1$. The average water requirement of the twenty duplicate cultures of this series for tops was 361 gm. In the ammonium sulfate series the corresponding values were 192 gm. for the lowest and 354 gm. for the highest, with cultures $T_1R_7C_1$ and $T_1R_1C_3$, respectively. Culture $T_1R_7C_1$ it should be remembered, gave the lowest yields of both tops and roots. The average water requirement of all the cultures of this series was 322 gm. The average value for both series was 342 gm., this value being taken as 100 in calculating the relative value for each culture.

The water requirement of the tops of the plants grown in the nutrient solution containing ammonium sulfate was considerably lower than the corresponding values for the plants grown in the solution of the Tottingham series. This is illustrated by figure 7, which also reveals the fact that the values of the water requirement for the two series run fairly parallel. Severely injured culture $T_1R_7C_1$ of the ammonium sulfate series is an exception to this general tendency. The curve for the Tottingham series was well above the other one.

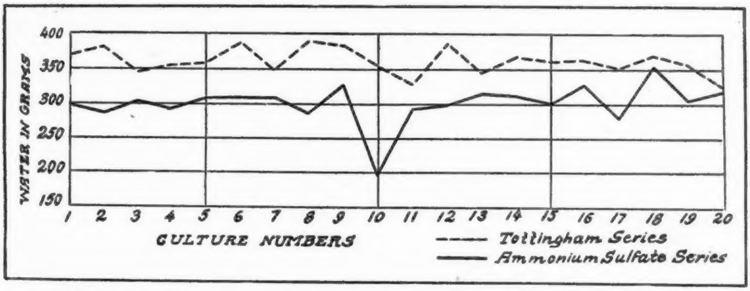

FIG. 7. WATER REQUIREMENTS IN GRAMS OF TOPS OF SOYBEANS PER GRAM OF DRY MATTER PRODUCED IN TOTTINGHAM AND AMMONIUM SULFATE SERIES

Again, as in the case of the yield of tops, the indication is clear that the nutrient solution containing ammonium sulfate, in comparison with the unmodified Tottingham solution, was better suited for the growth of soybeans.

In the experiments here recorded the total transpiration of water by the same number of plants in the ammonium sulfate series is sometimes more and sometimes less than in the case of Tottingham series, but it is always less per gram of dry weight of plants, so far as the yield of tops is concerned. No such consistent relation could be traced in the water requirement of the roots of the two series.

The water requirement of dry roots is, of course, many times as high as it is for tops. In the Tottingham series the values varied from 1580 gm. in culture $T_3R_1C_5$ to 2595 gm. in culture $T_1R_3C_3$, the average for all cultures being 2060 gm. The same values for the ammonium sulfate series were: lowest, 1840 gm. in culture $T_5R_1C_1$, and the highest, 2950 gm. in $T_1R_5C_3$, the average being 2.088 gm. The average water requirement of the roots for 40 duplicate

cultures was 2074 gm. Thus, the plants of the Tottingham series in the root development, as average figures show, were slightly more economical than the plants of the ammonium sulfate series.

COMPARISON OF YIELDS OF SOYBEANS GROWN IN NUTRIENT SOLUTIONS EMPLOYED BY OTHER AUTHORS

For the purpose of comparison, in the second trial of the experiment six additional cultures were prepared. Three duplicate cultures of the following solutions were employed: (a) Crone's, (b) Knop's and (c) Shive's. These

TABLE 8

Comparative growth of soybeans in sand cultures treated with nutrient solutions proposed by different authors

	YIELD OF TOPS		YIELD OF ROOTS		RELATIVE VALUE OF TOTAL YIELD	TRANSPIRATION		WATER REQUIREMENT OF TOPS		WATER REQUIREMENT OF ROOTS	
	Dry weight	Relative value	Dry weight	Relative value		Amount	Relative value	Per gram of dry matter	Relative value	Per grams of dry matter	Relative value
	grams		*gram*			*grams*					
Crone 1	2.4092		0.5120			760					
Crone 2	2.4050		0.5108			758					
Average	2.4071	83	0.5114	100	183	759	72	371	95	1484	71
Knop 1	1.7975		0.3387								
Knop 2	1.6758		0.3862			825					
Average	1.7367	60	0.3625	71	131	825	79	475	121	2276	109
Shive 1	2.7605		0.4312			1088					
Shive 2	2.5455		0.4563			1069					
Average	2.6530	92	0.4438	87	179	1079	102	407	104	2431	116
Tottingham	2.7285	96	0.4510	88	184	1028	98	404	103	2279	109
Ammonium sulfate series	5.2335	182	0.7948	155	337	1581	150	302	77	1989	95
Average for all cultures	2.8791	100	0.5127	100	200	1054	100	392	100	2092	100

nutrient solutions had the following partial volume-molecular concentrations, each with a total concentration approximating an osmotic value of 2.5 atmospheres:

Crone's (4) solution: KNO_3, 0.0392 m.; $Ca_3(PO_4)_2$, 0.00314 m.; $Fe_3 (PO_4)_2$, 0.0027 m.; $MgSO_4$, 0.008 m.; and $CaSO_4$, 0.007 m.

Knop's (13) solution: KNO_3, 0.0083 m.; KH_2PO_4, 0.0063 m.; $Ca(NO_3)_2$, 0.0207 m.; and $MgSO_4$, 0.0072 m.

Shive's (26) solution: KH_2PO_4, 0.0257 m.; $Ca(NO_3)_2$, 0.0074 m.; and $MgSO_4$, 0.0214 m.

The procedure of the experiment was exactly the same as in the main experiment. The results obtained with these nutrient solutions, together with

the data for the best yields from the Tottingham series and from the ammonium sulfate series, are presented in table 8.

An examination of the table 8 reveals the striking influence of the introduction of ammonium sulfate into Shive's nutrient solution, or of substituting it for KNO_3 in the Tottingham nutrient solution. The growth of soybeans in both the tops and the roots was very markedly increased.

Table 8 shows, in general, that transpiration was increased with the increase in growth. Also, the amount of water used per dry weight of crop was smaller in the case of plants of better development than in those of poor growth. On the whole, this is true of both the top and the root yields. Crone's nutrient solution deviated somewhat from this general rule.

DISCUSSION

In the foregoing experiments it was shown that ammonium sulfate, when added to Shive's nutrient solution, or when used as a substitute for potassium nitrate in Tottingham's nutrient solution, caused a greatly variable effect on the growth of both the tops and the roots of soybeans. In twenty representative cultures, of the 84 possible cultures used by Tottingham, ammonium sulfate in some salt proportions resulted in a very severe injury to the plants, while in other combinations it caused a remarkably high increase in the plant growth over the corresponding cultures of the Tottingham solution. Because of the complexity of the problem, it is often difficult (if not impossible) to determine why such profound differences occur in two cultures whose nutrient solutions differ but slightly. These questions were thoroughly discussed by Tottingham (29), Shive (26), and McCall (19) in the parallel cases and need not be repeated here. It is advisable, however, to trace the relations in connection with ammonium sulfate. Beginning with culture 21, or $T_1R_1C_1$, it will be observed (table 5) that with the smallest applications of mono-potassium phosphate, ammonium sulfate and calcium nitrate, when the partial osmotic concentrations supplied by magnesium sulfate was seven-tenths of the total concentration, the yield of the soybeans was well above the average. In the next culture, on the increase of calcium nitrate, the mono-potassium phosphate remaining the same, the yield increased about 13 per cent over the former. On the further increase of calcium nitrate and decrease in magnesium sulfate, the yield of tops did not increase, but was slightly decreased. A similar yield was obtained with the next culture having the highest application of calcium nitrate, although the roots (table 6) continued to increase. In culture 25, when the first increase in the amount of ammonium sulfate with the least amount of calcium nitrate was employed, the yield fell from 152 for culture 24 to 78 per cent for culture 25. The increase in calcium nitrate in the next culture to three-tenths of the total osmotic concentration brought the yield of plants to 132 per cent of the average. This concentration of calcium nitrate is the largest that could be safely used in combination with ammo-

nium sulfate in these experiments. In the next culture, No. 27, upon an increase in the calcium nitrate to five-tenths of the total osmotic concentration, a decrease in the yield of both tops and roots followed. The best yield of soybeans occurred in the culture with salt proportions as follows: 0.0156 m. mono-potassium phosphate, 0.0036 m. ammonium sulfate, 0.0108 m. calcium nitrate, and 0.0174 m. magnesium sulfate. On the reduction of calcium nitrate to one-tenth of the total osmotic concentration in culture 31, or on the increase of its proportion to five-tenths in culture 33, the decrease in the yield of tops was 30 per cent and 27 per cent, respectively. The difference effected in the case of yield of roots was still larger. It seems, therefore, that the development of the plant is the result of the combination, not of the different separate components of the nutrient solution. Tottingham (29), Shive (26), and later, McCall (19) have set forth the dependence of the separate salts upon the proportionality of the component factors in the solution, though the variation due to different salts in the instances of the above-mentioned investigators was not so pronounced as the variation due to difference in proportions of ammonium sulfate used in the present work.

It is commonly noticed that when an excess of ammonium sulfate is applied to an agricultural soil, an injury results to the plants grown therein. This injury varies according to the conditions under which the experiment is carried out. The amount of salt used, type of soil, amount of lime present (and also other fertilizers), moisture content, temperature, and kind of crop, are among the factors modifying the effect produced by ammonium sulfate in soil. In most cases, the ill effect of an excessive application of this salt is attributed to the acid production in the soil, when ammonium is used by the crop or converted into nitrate, leaving the acid radical behind. Another possibility, however, may be mentioned. It is a well established fact that there may be an injurious effect of a certain ion, as such, if its action is not antagonized by some other ion. McCool (20) reports that the NH_3-ion is very injurious above certain concentrations. Its ill effect, however, is diminished by the presence of other salts and can be completely destroyed by the antagonistic action of Ca or Na. In this connection it is interesting to note that the injurious effect of Na can be destroyed by the addition of a proper amount of NH_3. Though McCool dealt with the chlorides of these bases, the similar behavior of NH_3 in the form of sulfate can be expected. Although it is a common practice to correct the soil injured by excessive amounts of ammonium sulfate, with lime (CaO, $Ca(OH)_2$, $CaCO_3$, or $CaMg(CO_3)_2$), Schulze (28) in extensive field experiments counteracted the injurious effect of ammonium sulfate with the aid of sodium chloride, thus securing results similar to those which McCool obtained under better controlled laboratory conditions.

In certain salt proportions the ammonium sulfate is not only harmless to the development of the plants but extremely beneficial. Here lies the explanation of the fact that there are so many conflicting reports published regarding the effect produced by the action of this salt, when used as a fertilizer. It

seems that poorly balanced nutrient salt combinations account for the prejudice toward this salt as a source of nitrogen for plant growth.

That the differences caused by ammonium sulfate in the present experiments, observed from culture to culture, brought about by the change of salt proportions, were not due to the change in the concentration of the resultant solutions, is clearly brought out in table 3, which was previously discussed. Figure 3 also emphasizes the same point. The differences in the total osmotic concentration of the nutrient solution of the different salt proportions, in both the original form and after application to the sea sand, were not great enough to account for the large differences in the plant growth. Moreover, Ayres (1), working with tobacco in sand cultures, has shown that the total concentration, as measured by the total weight of salts in the initial application, is not as large a factor for the normal development of plants as the proper balance between the different salts applied. The preliminary work of McCall (19) also shows similar results.

CONCLUSION

In the experiments presented above, the work was done with soybeans grown in sand cultures.

The effect of ammonium sulfate on Shive's nutrient solution was studied. For this purpose ammonium sulfate was substituted for potassium nitrate in Tottingham's nutrient solution, which solution, as a control, was used in parallel with the ammonium sulfate series.

The osmotic concentration of the solutions was about 2.5 atmospheres, as calculated in advance.

The tested osmotic concentration of the sand in culture pots, using the freezing-point method, showed that the concentration of sand treated with the solution was nearly the same as that of the original solution.

With few exceptions all these values were below the calculated 2.5 atmospheres.

There was considerable difference between the action of Tottingham's nutrient solution and that containing ammonium sulfate in the place of potassium nitrate. In the case of the Tottingham nutrient solution the concentration of sand treated with solution in the laboratory was lower than the concentration of the original solution. On the other hand, in the sand treated with the nutrient solution containing ammonium sulfate, the concentration, with few exceptions, was higher than the concentration of the corresponding original solutions.

These phenomena were attributed to selective adsorption of ammonia, which, leaving the acid radical behind, resulted in the formation of acid, which later became more ionized than its salt, thus causing a greater depression of the freezing-point than could be expected from the salt solution.

The concentration values of the solutions, obtained by suction from the

pots at different periods of the experiment, did not vary considerably from those of the original solution. This indicated that the adsorption by sand was not the factor that could modify the concentration of the solution to such an extent as to cause even slight differences in plant growth.

Ammonium sulfate, when substituted for potassium nitrate, resulted in a better yield of soybeans in certain salt proportions, than in the corresponding salt proportions with potassium nitrate, but caused considerable injury when added in excess.

The foliage of the plants receiving ammonium sulfate, on the whole, had a greener color than that of the plants in the Tottingham series.

The best cultures for the growth of tops in the Tottingham series were $T_3R_1C_1$ and $T_3R_1C_3$, corresponding to 0.0156 m. mono-potassium phosphate, 0.0049 m. potassium nitrate, 0.0036 m. calcium nitrate, and 0.0290 m. magnesium sulfate in the former, and 0.0156 m. mono-potassium phosphate, 0.0049 m. potassium nitrate, 0.0108 m. calcium nitrate and 0.0174 m. magnesium sulfate in the latter.

The best cultures of the ammonium sulfate series was $T_3R_1C_3$, which contained 0.0156 m. mono-potassium phosphate, 0.0036 m. ammonium sulfate, 0.0188 m. calcium nitrate and 0.0174 m. magnesium sulfate.

The best culture of the ammonium sulfate series gave a dry weight of tops 35 per cent higher than that of the best culture in the Tottingham series.

In the case of top yields, only two cultures in the Tottingham series gave results 110 per cent or more of the average, while twelve cultures gave 90 per cent or less than 90 per cent of the average. In the ammonium sulfate series, twelve cultures were above and five cultures below this average.

The variations from culture to culture with the change in the salt proportions were much greater in the ammonium sulfate series than in the Tottingham series.

Similar relations exist, in general, in the case of root development. However, more cultures in the ammonium sulfate series gave root yields below 90 per cent of the average than the number of cultures in the same series that gave top yields below 90 per cent of the average.

There is a very close relation between the yield of tops, yield of roots, and the total transpiration of the plants. Increase in the yield of tops was followed by an increase in the yield of roots, and both these values were accompanied by increased transpiration.

The water requirement (water required to produce 1 gm. of dry matter) of soybean tops in the cultures of the ammonium sulfate series was less than that in the corresponding cultures of the Tottingham series.

In comparing the growth of soybeans in sand cultures treated with different solutions, as proposed by other authors, the order of magnitude of yields of tops was as follows: ammonium sulfate series > Tottingham > Shive R_5C_2 > Crone > Knop.

The author wishes to acknowledge his indebtedness to Dr. John W. Shive

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CARBONIC ACID GAS IN RELATION TO SOIL ACIDITY CHANGES

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During the past two years four articles have been published which deal with certain phases of recent work on soil acidity. The articles are:—"Acid Soils and the Effect of Acid Phosphate upon Them" by S. D. Conner; (2) "Acidity and Absorption as Measured by the Hydrogen Electrode," by Sharp and Hoagland (6); "The Reaction of Soil and Measurements of Hydrogen Ion Concentration" by Gillespie (5) and "Liming and Lime Requirement of Soil," by Ames and Scholenberger (1). Among the recent workers on and contributors to theories of soil acidity may be mentioned Cornu, Daikuhara, Frear, Gans, Harris, MacIntire, Morse, Parker, Rice, Ruprecht, Saidel, Sullivan, Truog and Veitch.

Acidity of soils is corrected by additions of lime. One of the most generally recommended forms of lime to apply is finely-ground calcium limestone containing as high as 44 per cent of carbon dioxide. All good agricultural practices involve the addition of organic matter to the soil. Soil organic matter is decomposed by bacteria with the production of immense amounts of carbon dioxide, and thus the effect of carbon dioxide upon soil acidity is worthy of investigation. The work of Coville (3, 4) and others has shown that organic matter is acid in reaction at certain stages of its decay. We are aware of no work that absolutely proves that this acidity is other than that due to carbonic acid weakly held by the organic matter.

The experiments reported upon here were designed primarily to obtain data on the effect of carbon dioxide additions to soil in relation to soil and plant

*Reaction of minerals after extraction by carbon dioxide solution**

ROCK OR MINERAL	ORIGINAL REACTION	REACTION AFTER TREATMENT WITH CO_2	
		With water	After addition of KCl solution
Granite	Alkaline	Weakly acid	Weakly acid
Gneiss	Alkaline	Weakly acid	Acid
Hornblende Andesite	Weakly alkaline	Weakly acid	Acid
Basalt	Strongly alkaline	Weakly acid	Weakly acid
Feldspar	Strongly alkaline	Weakly acid	Weakly acid
Mica	Strongly alkaline	Acid	Acid

* From Ames, J. W. and Schollenberger, C. J. (1, p. 331).

changes. The acidity data obtained were reported in a separate paper since they give additional information confirmatory of the work of Daikuhara which has been quoted in many articles on soil acidity.

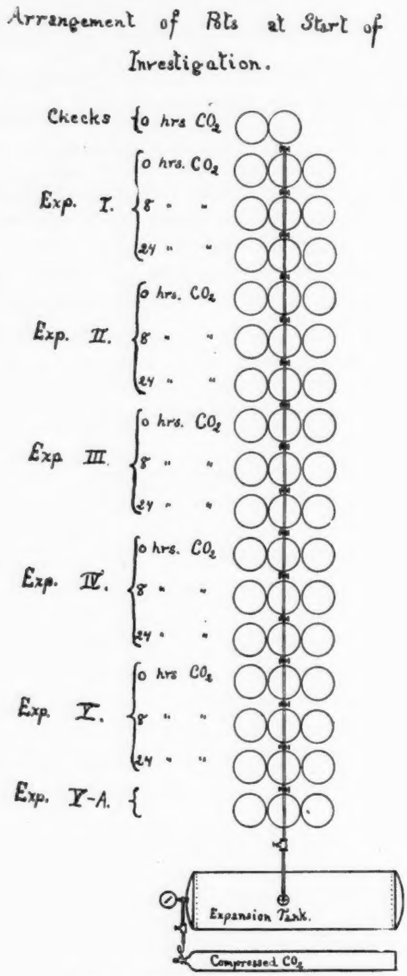

FIG. 1. SYSTEM OF TREATMENT

PLAN OF THE INVESTIGATION

Equal weights of soil were put into paraffined Wagner pots of the most approved type, after the soil had been thoroughly mixed and specified applications of fertilizer and lime added. The soil was compacted uniformly in

the pots by dropping them a prescribed number of times. Distilled water was added to bring the moisture content up to half saturation and small pepper plants were transplanted into the pots. The soil was kept with a dust mulch and uniform moisture content throughout the entire investigation, extending from February 4 to December 5, 1916. The position of the pots was changed from time to time in such a way that each pot was in a position occupied by every other pot at least twice during the period of investigation.

In each experiment there were nine pots—three which received no carbon dioxide application, three where the gas bubbled into the soil between 8.00 a.m. and 4.00 p.m. and three where the gas was bubbled constantly into the soil. Figure 1 shows the arrangement of the different experiments on the

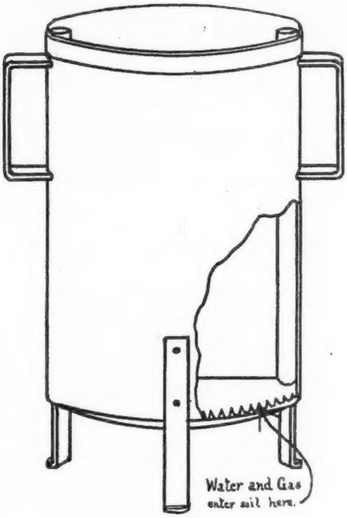

Fig. 2. Wagner Pot

greenhouse bench, figure 2 shows a Wagner pot. A set of pots is shown in plate 1. Carbon dioxide applications were made to the soil from April 16 to the close of the investigation. The gas was applied, to each pot at the rate of approximately 650 cc. (under standard conditions) of gas per hour of treatment given.

ACIDITY METHODS USED

In making acidity tests two methods were used—that recommended by Veitch and that recommended by Hopkins, Pettit and Knox. In one experiment lime was added to satisfy the lime requirement as determined by the Veitch method; in another case one-half this amount was used; and in a third instance one and one-half times the lime requirement was applied. In both

methods special care was taken in titrating. The potassium nitrate used for the Hopkins method was neutral to phenolphthalein.

Analysis of soil

	Per cent
Insoluble residue (1)	82.05
K_2O (1)	0.41
Na_2O (1)	0.36
CaO (1)	0.44
MgO (1)	0.79
Fe_2O_3 (2)	4.35
Al_2O_3 (1)	3.65
P_2O_5 (3)	0.13
SO_2 (1)	0.52
Water (4)	2.31
Volatile matter (4)	5.76
Nitrogen (5)	0.19
Total carbon (6)	2.12
Inorganic carbon (7)	0.03

(1) Solution and residue made by extracting soil on steam bath with hydrochloric acid (specific gravity 1.115) for 10 hours. Solution analyzed by usual procedures.

(2) Determined volumetrically from acid solution with hydrogen sulfide as reducing agent.

(3) Method of Goss.

(4) Five-gram aliquot used and both determinations made on same aliquots.

(5) Regular Kjeldahl.

(6) Method of Ames and Gaither as modified by Schollenberger.

(7) Method of Marr with dilute acid and vacuum as recommended by Ames of Ohio.

Table 1 and figure 3 give the soil acidity as influenced by cropping, fertilization, lime, and carbon dioxide. The figures express parts of calcium carbonate per hundred parts of dry soil as determined by the Hopkins potassium nitrate method. It is noted that the acid soil increases in acidity when allowed to stand in the greenhouse with its water-holding capacity one-half satisfied. The increase in acidity of 350 pounds per million pounds of soil was more than the acidity of the soil at the start of the investigation. When the soil was cropped in experiment 1 an increase in acidity, slightly greater than where there was no crop, resulted. The carbon dioxide applications increased the acidity further. The constant treatment of carbon dioxide gave the greater increase in acidity.

In experiment 2 we note that the lime without carbon dioxide gas prevented as large an increase in acidity, whereas carbon dioxide gas applications with lime gave greater increases in acidity.

In experiment 3 the soil was fully neutralized according to the Veitch method, but gave almost as great an increase in acidity as where one application of lime was used. The extra application of lime prevented the increases in acidity due to carbon dioxide treatments from being as great.

In experiment 4 the triple application of lime and one application of phosphorus gave smaller acidity increases where no carbon dioxide was applied.

TABLE I

*Soil acidity as influenced by cropping, fertilization, liming and carbon dioxide**

	CO_2 TREATMENT GIVEN PER DAY	$CaCO_3$ ADDED	ACIDITY AT END OF INVESTIGATION (HOPKINS METHOD)	TOTAL INCREASE IN ACIDITY (HOPKINS METHOD)	INCREASE DUE TO CO_2
	hours				
Acid soil, no crop	0	0.0000*	0.0583*	0.0350*	
Experiment 1					
Acid soil, with crop	0	0.0000	0.0592	0.0359	
	8	0.0000	0.0716	0.0483	0.0124†
	24	0.0000	0.0881	0.0648	0.0289
Experiment 2					
Acid soil, with crop, with single application of $CaCO_3$	0	0.0770	0.0362	0.0129	
	8	0.0770	0.0644	0.0411	0.0282
	24	0.0770	0.0736	0.0503	0.0374
Experiment 3					
Acid soil, with crop, with double application of $CaCO_3$	0	0.1540	0.0353	0.0120	
	8	0.1540	0.0449	0.0216	0.0092
	24	0.1540	0.0510	0.0277	0.0157
Experiment 4†					
Acid soil, with crop, with triple application of $CaCO_3$ and single application of phosphorus Banner bone (di-calcium phosphate)	0	0.2310	0.0242	0.0009	
	8	0.2310	0.0350	0.0117	0.0108
	24	0.2310	0.0333	0.0100	0.0091
Acid phosphate	0	0.2310	0.0324	0.0091	
	8	0.2310	0.0456	0.0223	0.0132
	24	0.2310	0.0416	0.0183	0.0092
Experiment 5‡					
Acid soil, with crop, with triple application of $CaCO_3$ and single application of nitrogen Dried blood	0	0.2310	0.0315	0.0082	
	8	0.2310	0.0397	0.0164	0.0082
	24	0.2310	0.0454	0.0221	0.0139
Sodium nitrate	0	0.2310	0.0321	0.0088	
	8	0.2310	0.0321	0.0088	0.0000
	24	0.2310	0.0475	0.0242	0.0154

TABLE 1—(Continued)

Experiment 5A §

	CO_2 TREATMENT GIVEN PER DAY	$CaCO_3$ ADDED	ACIDITY AT END OF INVESTIGATION (HOPKINS METHOD)	TOTAL INCREASE IN ACIDITY (HOPKINS METHOD)	INCREASE DUE TO CO_2
Acid soil, with crop, with triple application of $CaCO_3$ and approximately one-half application of nitrogen in form of sodium nitrate	0	0.2310	0.0227	0.0004	
	8	0.2310	0.0318	0.0085	0.0081
	24	0.2310	0.0365	0.0132	0.0128

* Single application of lime equals 770 parts $CaCO_3$ per million of dry soil.

Single application of phosphorus equals 501.5 parts per million of dry soil.

Single application of nitrogen equals 235.6 parts per million of dry soil.

Acidity of soil used for investigation 0.0233 (by Hopkins) and 0.1540 (by Veitch) parts of $CaCO_3$ per 100 parts of dry soil.

† Figures express parts $CaCO_3$ per 100 parts of dry soil.

‡ In experiments 4 and 5 comparisons were made between different carriers of phosphorus and nitrogen.

Two pots in each set of three received Banner Bone and one acid phosphate in experiment 4.

Two parts in each set of three received dried blood and one sodium nitrate in experiment 5.

§ CO_2 treatment started 20 weeks later than in other experiments.

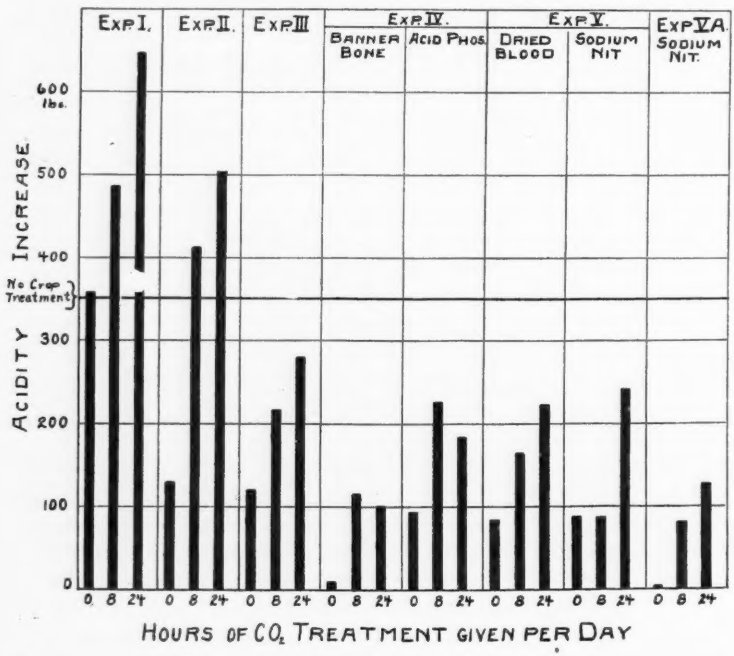

FIG. 3. ACIDITY CHANGES DUE TO CROPPING, LIMING, FERTILIZATION AND CARBON DIOXIDE TREATMENTS

This might have been due to the extra application of calcium carbonate. The increases in acidity with Banner Bone were much less than where acid phosphate was used. The increases in acidity due to carbon dioxide applications were less with both phosphorus carriers under constant carbon dioxide treatments than they were with the intermittent carbon dioxide treatments. The increases due to carbon dioxide were practically the same with Banner Bone as they were with acid phosphate.

The effects of carbon dioxide in increasing soil acidity where dried blood and sodium nitrate were compared as nitrogen carriers are given under experiment 5. With no carbon dioxide treatment the increases in soil acidity were practically the same for dried blood and sodium nitrate. With the intermittent carbon dioxide treatment there was practically double the total increase with dried blood that there was with sodium nitrate. With the constant carbon dioxide treatment the total increase was slightly greater where sodium nitrate was used. This made the increases due to carbon dioxide practically the same where constant carbon dioxide treatments were given, but no increase and 82 pounds increase with the intermittent gas treatments.

Experiment 5-A is a test of one pot in each case to determine if results would be greatly different if smaller amounts of sodium nitrate were used. It is noted that there is practically no increase in acidity without the carbon dioxide treatments but that acidity is increased by both carbon dioxide applications.

Figure 4 gives the acidity titrations in cubic centimeters of N/10 sodium hydroxide in relation to the weights of ignited precipitates from the acidity solutions. These precipitates were obtained from the hot solutions by adding ammonium hydroxide after the sodium hydroxide precipitates had been dissolved with hydrochloric acid and the solutions boiled. This graph would be a continuous curve or straight line instead of an oscillating graph if the treatments given this soil changed its acidity in proportion as the potassium-nitrate-soluble iron and aluminum changed. The graph shows that the Hopkins potassium nitrate method gives acidity results that can not be correlated with the amount of aluminum in the soil that is in solution in presence of a normal salt of a strong acid and strong base.

The ignited precipitates were composited by carbon dioxide treatments so that the effect of carbon dioxide applications on the composition of the precipitate could be determined. Weights and analyses of precipitates are given in table 2.

There are many theories as to the nature of soil acidity, which may be classified in two groups: physical and chemical. Among the physical theories we have one depending upon what has been termed "selective adsorption," where the soil is supposed to adsorb the basic portion of the compound. Of the chemical theories offered we find an explanation based on the existence of free acids in soils as the resultant of hydrogen-ion determinations and mass-action studies. In fact, some chemical theories of soil acidity take into account the relative velocity of different chemical reactions. The data given

TABLE 2

Weights and analyses of ignited precipitates

	CARBON DIOXIDE TREATMENT PER DAY		
	0 hours	8 hours	24 hours
Total weight of precipitate analyzed (gm.)	0.2129*	0.2331	0.2362
Silicon dioxide—SiO_2 (per cent)	9.34	7.51	8.68
Ferric oxide—Fe_2O_3 (per cent)	1.32	1.09	1.28
Aluminum oxide—Al_2O_3 (by difference) (per cent)	89.34	91.40	90.04

* Includes precipitate from pot receiving no crop and no CO_2 treatments.

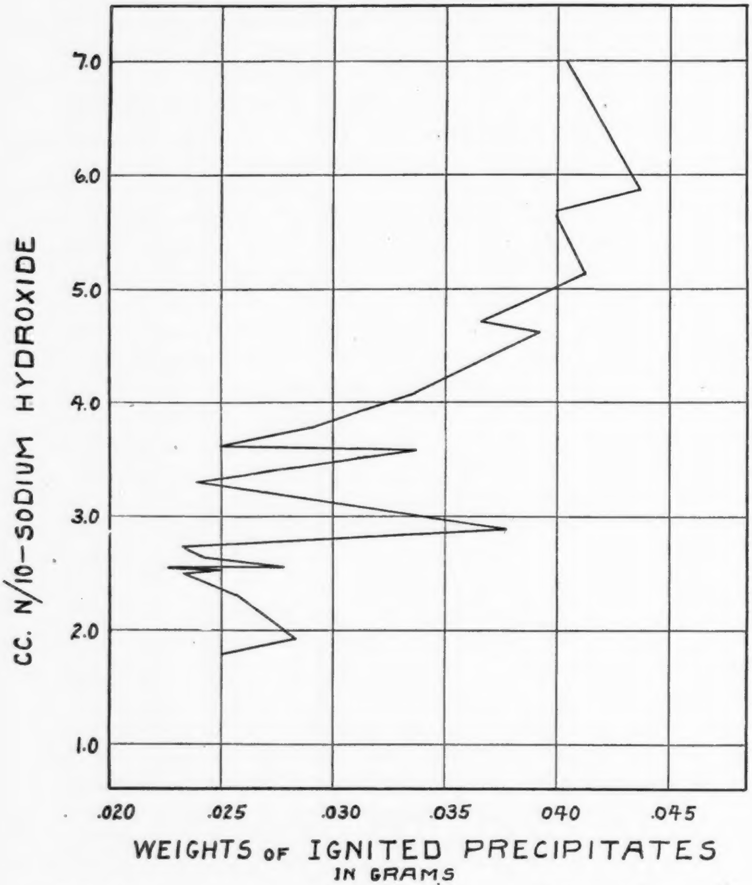

FIG. 4. RELATION BETWEEN ACIDITY TITRATIONS AND WEIGHT OF IGNITED MATERIAL PRECIPITATED FROM ACIDITY SOLUTIONS BY AMMONIUM HYDROXIDE

in this paper support the chemical theories of soil acidity, inasmuch as different applications of a gas of definite composition (carbon dioxide), which is not only soluble in water but which combines with it, yielding hydrogen ions, caused differences in soil acidity. These differences in soil acidity have varied with different fertilizer and lime applications, both with and without carbon dioxide treatments. This leads us to conclude that the figures given by the Hopkins potassium nitrate method show that liming, fertilization, and carbon dioxide applications, both alone and in combination, have brought about different chemical reactions in the soil used.

CONCLUSIONS

1. Keeping soil at one-half its water-holding capacity in a greenhouse increased its acidity.
2. Cropping soil kept at one-half its water-holding capacity increased its acidity.
3. The increases in acidity of cropped soil were modified by different applications of calcium carbonate.
4. The increases in soil acidity of cropped soil varied with different fertilizer applications.
5. Carbon dioxide added to cropped soil, treated with lime alone or lime and fertilizer, increased its acidity.
6. The results of these experiments support chemical theories as to the nature and causes of soil acidity.
7. The changed reactions of this soil towards a neutral salt of a strong acid and a strong acid (KNO_3) after subjection to the varied conditions of the experiment at least suggests that soil acidity is largely the result of hydrolytic mass action phenomena.

The authors wish to make acknowledgment to Director C. G. Woodbury for permission to carry on this work.

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PLATE I

Pots Connected with Carbon Dioxide Line for Carbon Dioxide Applications to Soil


25

THE NITROGEN DISTRIBUTION OF FIBRIN HYDROLYZED IN THE PRESENCE OF FERRIC CHLORIDE

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INTRODUCTION

In a recent investigation of the distribution of the organic nitrogen in the soil Morrow and Gortner (5) made the statement that "the humin nitrogen of protein origin actually present in the hydrolyzed soil may easily be a very small part of the nitrogen found." It was shown that from 3.26 to 9.21 per cent of the total nitrogen was precipitated by calcium hydroxide. This did not represent *true* humin nitrogen since the calcium hydroxide precipitate did not contain any black substance formed by hydrolysis. The solution from which it was precipitated was colored only with ferric compounds. They therefore concluded that the organic material in this precipitate must consist of colorless organic compounds absorbed by or combined with the lime and that the nitrogen contained in this fraction must consist almost entirely of material of non-protein origin since in pure proteins the nitrogen retained in the calcium hydroxide precipitate is supposed to consist entirely of deeply colored substances.

Gortner (2) has shown that when fibrin is hydrolyzed in the presence of varying amounts of carbohydrates the humin nitrogen shows an increase. This indicates that a pure protein when hydrolyzed in the presence of an oxidizing agent such as a carbohydrate causes a redistribution of the fractions with an increase in the humin nitrogen.

EXPERIMENTAL

The problem

We have made a study of the distribution of the nitrogen of a pure protein hydrolyzed in the presence of ferric chloride as an oxidizing agent. This compound was chosen because all mineral soils contain compounds of iron and it was thought that additional data might be obtained which would throw more light on the formation of humin nitrogen.

The material

The study has been made on a pure protein. The protein selected was Merck's fibrin from blood.

The method

Duplicate analyses were made with fibrin and fibrin plus ferric chloride. In each case 3 gm. of fibrin were hydrolyzed in the presence of HCl (sp. gr. 1.115) for 48 hours at gentle boiling. The calculated amount of hydrated ferric chloride equivalent to 30 gm. of anhydrous ferric chloride was added to one set of duplicates. The calculated amount of concentrated hydrochloric acid was also added to the flasks containing the ferric chloride, so that the

TABLE 1

Comparative analyses of 3 gm. of fibrin hydrolyzed alone and in the presence of 30 gm. of anhydrous ferric chloride

NITROGEN	3 GM. FIBRIN, NO $FeCl_3$					3 GM. FIBRIN + 30 GM. $FeCl_3$				
	Nitrogen (mgm.)		Per cent of total N			Nitrogen (mgm.)		Per cent of total N		
	I	II	I	II	Average	I	II	I	II	Average
Total	0.4538	0.4444				0.4546	0.4617			
Ammonia	0.0473	0.0468	10.42	10.53	10.48	0.0589	0.0618	12.95	13.39	13.17
Acid-insoluble humin	0.0095	0.0096	2.09	2.16	2.13	0.0075	0.0077	1.65	1.67	1.66
Acid-soluble humin precipitated by $Ca(OH)_2$	0.0047	0.0043	1.04	0.97	1.00	0.0459	0.0443	10.09	9.59	9.84
Phosphotungstic acid humin	0.0010	0.0046	0.22	1.04	0.64	0.0046	0.0036	1.01	0.78	0.90
Total humin	0.0152	0.0185	3.35	4.16	3.76	0.0580	0.0556	12.76	12.04	12.40
Basic	0.1143	0.1230	25.19	27.68	26.44	0.1114	0.1072	24.50	23.21	23.86
Arginine	0.0627	0.0598	13.81	13.46	13.63	0.0615	0.0605	13.53	13.10	13.31
Histidine	None	0.0023	None	0.52	0.26	None	None			None
Lysine	0.0499	0.0585	11.00	13.16	12.08	0.0464	0.0434	10.21	9.40	9.81
Cystine	0.0017	0.0024	0.37	0.54	0.46	0.0035	0.0033	0.77	0.71	0.74
Amino, in bases	0.0700	0.0673	15.43	15.15	15.29	0.0688	0.0709	15.13	15.36	15.25
Non-amino, in bases	0.0443	0.0557	9.76	12.53	11.15	0.0426	0.0363	9.37	7.86	8.61
Filtrate from bases	0.2726	0.2671	60.06	60.10	60.08	0.2346	0.2333	51.59	50.54	51.07
Amino, in filtrate from bases	0.2500	0.2512	55.08	56.52	55.80	0.2077	0.2123	45.68	45.97	45.82
Non-amino, in filtrate from bases.	0.0226	0.0159	4.98	3.58	4.28	0.0269	0.0210	5.92	4.55	5.24
Total recovered	0.4494	0.4554	99.02	102.48	100.75	0.4629	0.4579	101.85	99.17	100.51

water of crystallization in the salt would not reduce the acid below constant boiling. The resulting hydrolysates were analyzed according to Van Slyke's (6, 7) method. Instead of the single humin nitrogen fraction we have found it desirable to follow the suggestion of Gortner and Holm (3) and separate the humin nitrogen into three fractions (a) acid-insoluble humin, (b) acid-soluble humin [precipitated by $Ca(OH)_2$] and (c) phosphotungstic acid humin (precipitated by phosphotungstic acid).

The "acid-insoluble humin" was obtained by first diluting the cold hydrolystate with about an equal volume of water, filtering off the insoluble humin and washing until free of chlorides. A Kjeldahl determination gave the nitrogen content. After the removal of the acid-insoluble humin the acid filtrate was evaporated under diminished pressure until all the hydrochloric acid possible was driven off. Water, alcohol and a suspension of calcium hydroxide were added and the ammonia determined in the usual manner. In the determination of the ammonia nitrogen from the fibrin plus ferric chloride, however, the distillation was continued for an hour, since it has been shown by one of us (5) that all the ammonia nitrogen was not driven off in a half-hour when the volume of the solution was large and a bulky precipitate of hydroxide was present. The precipitate remaining in the distilling flask was filtered and washed free of chlorides. The samples to which ferric chloride was added were washed by decantation after the method previously described (5) for mineral soils. A Kjeldahl determination was made and the results recorded as "acid-soluble humin." The filtrate from the acid-soluble humin was concentrated and diluted to 250 cc. volume. Two 25-cc. portions were used for the determination of the total nitrogen in the solution. The remaining 200 cc. of the solution were used for the precipitation of the diamino acids. The precipitation, washing and the decomposition were carried out according to Van Slyke's directions. The washed precipitate of barium phosphotungstate was subjected to Kjeldahl analysis and the nitrogen was recorded as "phosphotungstic acid humin." The remainder of the analysis was completed as directed by Van Slyke. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without the necessity of a calculation.

Analytical data

The distribution of the nitrogen both as regards grams of nitrogen in the different fractions and the percentages of the total nitrogen is shown in table 1.

DISCUSSION

It is observed from a study of the table that the hydrolysis carried out in the presence of ferric chloride gives a larger proportion of the nitrogen in the fraction precipitated by calcium hydroxide than with the fibrin hydrolyzed alone. This increase in the acid-soluble humin is of the same order as that previously found for the hydrolysate of soils and in this instance corresponds very closely to the decrease in the filtrate from the bases.

In all probability the humin nitrogen precipitated by the calcium hydroxide from the soil hydrolysates was largely protein nitrogen instead of being of non-protein origin. There is no doubt, however, that some of this fraction must be due to non-protein material. Gortner (1) has shown that uric acid

nitrogen is distributed in all four of the major fractions after hydrolysis. Purine and pyrimidine bases are undoubtedly present in the soil organisms and a certain part of their nitrogen would be precipitated in the fraction under discussion.

It seems evident from this investigation that the earlier conclusions of one of us (5) in regard to the humin nitrogen precipitated by calcium hydroxide from the soil hydrolysate were in part in error, and that in all probability a part of these colorless substances are derived from the filtrate from the bases.

The origin of this acid-soluble humin derived from the filtrate from the bases may be due to one specific amino acid. It is known that ferric chloride oxidizes phenols, so that it is possible that the phenol group in tyrosine has reacted here.

The ammonia nitrogen fraction is increased when fibrin is hydrolyzed in the presence of ferric chloride. This is to be expected since the presence of ferric chloride elevates the boiling point of the liquid during hydrolysis. It has been shown by Henriques and Gjaldbaek (4) and Van Slyke (8) that when hydrolysis is carried out at a high temperature there is a transformation of some amino nitrogen into ammonia, indicating a deamination of some amino acids.

As has been stated above, Gortner (2) has shown that if the weight of carbohydrate material present during protein hydrolysis greatly exceeds the amount of protein, an accurate nitrogen determination cannot be obtained. *We have also shown that an exact determination of the chemical groups in proteins cannot be expected when proteins are hydrolyzed in the presence of ferric chloride.*

These results have an important bearing on the application of Van Slyke's method to soils. They would both be reactive in a soil hydrolysate and thus give erroneous values for nitrogen distribution even if all the nitrogen of the soils were contained in the form of protein. If we add to this the fact that non-protein nitrogenous materials are present, it is evident that the nitrogen distribution obtained on a soil hydrolysate has no relationship to those obtained on pure protein material.

SUMMARY

1. When a protein is hydrolyzed in the presence of ferric chloride an accurate nitrogen distribution cannot be obtained.

2. There is a substantial increase in the ammonia nitrogen when hydrolysis is carried out in the presence of ferric chloride. This is due to deamination of some amino acids at the temperature of hydrolysis.

3. The acid-soluble humin nitrogen increases at the expense of a corresponding loss in the filtrate from the bases.

4. This investigation indicates that the earlier conclusion in regard to humin nitrogen precipitated by calcium hydroxide is incorrect. A part of this acid-soluble humin is of protein origin instead of being largely non-protein.

5. This study emphasizes the fact that much of the recent work on the organic nitrogen distribution in soils by the Van Slyke method is entirely unreliable. The data cannot in any way represent the distribution of protein nitrogen in the soil.

It is the intention of one of us to test the suggestion experimentally in regard to the origin of the acid-soluble humin nitrogen as soon as possible.

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